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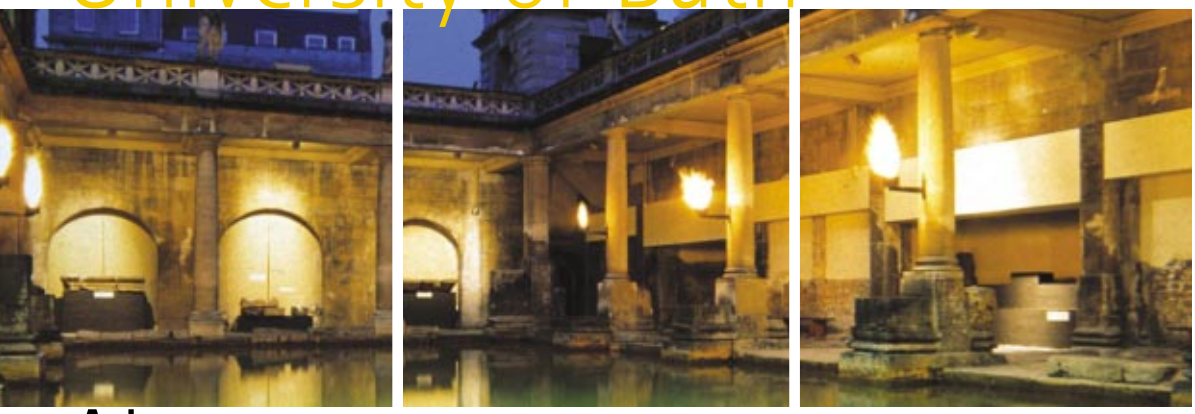

Abstracts

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Plenary session

Microbe–vector interactions in vector-borne diseases

Vector-borne diseases

Brian W.J. Mahy

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Vector borne infectious diseases are usually spread by arthropods, which may transmit helminths, protozoa, bacteria, rickettsia or viruses to cause major epidemics of human diseases such as Chagas disease, malaria, plague, trypanosomiasis, leishmaniasis, louse-borne typhus, dengue fever, yellow fever, Japanese encephalitis and West Nile fever. Malaria alone causes 300–500 million clinical cases and approximately 1.2 million deaths worldwide each year. Many of these diseases have been emerging in human populations in the last ten years as a result of failure to control mosquito populations, due in part to public demand for the withdrawal of DDT for environmental reasons, and the breakdown of public health infrastructure in developing countries which has led to poor vaccination coverage for diseases such as yellow fever, for which an excellent vaccine is available. In addition, changes in the structure and movement of human populations in recent years has led to the introduction of new pathogens into geographical areas previously considered free of the disease, as occurred in 1999 when West Nile virus entered the USA from Israel, and is now established in the American continent.

The principle features of vector borne diseases and new strategies for their control, such as approaches to control of Chagas disease and filariasis directed at essential symbiotic bacteria, will be discussed.

Evolution of tick-borne disease systems

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I shall argue that the evolution of tick-borne pathogens is *driven* by intrinsic biological barriers, but is *directed* and *constrained* by extrinsic environmental factors. This is illustrated by flaviviruses of the tick-borne encephalitis complex, contrasted with bacteria of the *Borrelia burgdorferi* s.l. complex. Tick vectors feed only once per life stage, which prolongs pathogen transmission cycles and probably accounts for the slow evolutionary change amongst tick-borne viruses. Vertebrate hosts have selected for different viral genotypes: flaviviruses of three distinct clades are transmitted via seabirds, via forest rodents and via sheep or goats. The mammal-associated lineages evolved as an east-west cline across the northern hemisphere. Despite the clade having colonised a large proportion of this hemisphere, each virus occupies defined foci within the wide, continuous ranges of its vector and host species. Persistent cycles of Western tick-borne encephalitis (WTBE) virus, for example, depend on specific climatic conditions, which have been related to the biological basis of transmission via rodents. Viruses transmissible via sheep or goats have been widely disseminated by livestock movement, but each new virus type has remained isolated, perhaps 'trapped' by specific environmental requirements. To test whether climate has been important in directing virus evolution, we are seeking correlations between virus phylogenetic trees and phenetic trees of the eco-climatic conditions in which each virus circulates. *B. burgdorferi* s.l. also shows evidence of selection by intrinsic vertebrate host factors, but environmental factors impose less constraint.

Keywords: Tick-borne flaviviruses, TBE, Louping ill, *Borrelia burgdorferi s.l.*, climate, host relationships

Insect transmission of viruses

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Viruses have evolved an impressive diversity of strategies for transferring efficiently form one host to the next. Organisms feeding on infected hosts and actively travelling in between hosts in the environment are all putative means of virus transport. Such organisms, designated vectors, are found among fungi, nematodes and arthropods, particularly insects. Several different interaction patterns have evolved between viruses and vectors, and this diversity is well illustrated in plant viruses transmitted by insects. Over half a century, a tremendous amount of studies has been carried out on the insect transmission of viruses, and a classification of the strategies observed has been established and repeatedly updated. This classification, originally established for the insect transmission of plant viruses, is comprehensive enough to illustrate all strategies described so far for virus–vector interactions, including animal viruses and the cases of transmission by non-insect vectors such as mites, ticks and nematodes. This chapter presents an overview of this classification, illustrated by appropriate examples. In addition, the fact that different categories or strategies of transmission are differentially represented in animal and plant viruses is highlighted and discussed.

RNA-based immunity in insects

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Drosophila has been an excellent model for the mechanistic studies of innate immunity. Recently, a new RNA-based antiviral immunity with features of both innate and adaptive immunities has been described in *Drosophila* and *Anopheles* cells. This RNA silencing-mediated immunity is characterized by the production of pathogen-derived, 22-nucleotide small RNAs that serve as specificity determinants inside a multi-subunit complex. Similar to innate immunity, however, the new invertebrate antiviral response is capable of a rapid virus clearance in absence of a virus-encoded suppressor of RNA silencing. The discovery of a new antiviral pathway in insects opens up the possibility of using this pathway to prevent transmission of vector-borne virus pathogens such as dengue and West Nile viruses.

Reducing the prevalence of *Borrelia* in ticks

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Abstract not received

Bunyavirus / mosquito interactions

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Bunyaviruses represent the largest group of arthropod transmitted viruses. They are characterised by a tripartite, negative-sense RNA genome that is replicated in the cytoplasm, and virion maturation

occurs in the Golgi. Many species are transmitted by mosquitoes, and the mosquito host plays a major role in the biological cycle of the virus. Mosquitoes function as over-wintering hosts and an important vessel for virus evolution, particularly through genome segment reassortment. Bunyaviruses do not cause any apparent damage to the mosquito though replicate with high efficiency; in contrast, they cause disease in mammalian hosts. This can be mimicked in the laboratory where infection of cultured mosquito cells is productive but no shut-off of host cell functions is detected, and the cells survive the infection though become persistently infected. In mammalian cells, however, host protein synthesis is shut-off and the cells are killed. To investigate the molecular interactions in more detail a reverse genetic system has been established in mosquito cells that allows dissection of bunyavirus RNA synthesis and other replication events. A recent finding is that a viral non-structural protein behaves quite differently in cells of disparate phylogeny, and could be the key to the observed differences in infection outcome.

How do mosquito vectors live with their viruses?

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There are at least 537 named arthropod-borne viruses (arboviruses), many of which have recently emerged or reemerged as an increasing threat to human and animal health. Although a wide variety of arthropods are vectors, this presentation focuses on mosquitoes. Despite over 100 years of research we still do not understand fully the relationship between arthropods and arboviruses. The specifics of the relationship between a particular mosquito species and an arbovirus are influenced by three factors: vector genetics, virus genetics and environmental conditions. The virus–vector relationship generally consists of a number of stages. (1) The mosquito must feed on a viraemic host. (2) An infectious dose of virus must enter the mosquito midgut lumen. (3) Virions must bind to the membrane of midgut epithelial cells. (4) Following endocytosis or fusion of the viral envelope and cellular membranes, the virus genome must enter the cell cytoplasm and replicate to produce infectious virions. (5) Virions must disseminate from the midgut epithelial cells and enter the haemocoel.(6) Virions must infect salivary glands. (7) Virions must be secreted in saliva when the mosquito feeds upon a host. These stages and factors that influence them will be discussed.

Environmental influences on arboviral infections and vectors

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In the context of climate-change those components of climate that are likely to have major effects upon distribution, seasonal incidence, abundance and prevalence of vector-borne diseases are described. On the basis of a predicted, global mean temperature increase in the order of 1.4 to 5.8°C, examples are provided of the sort of changes that may be expected by drawing upon recent laboratory work on the OIE List A diseases African horse sickness and bluetongue. Certain recent and dramatic alterations in the epidemiology of bluetongue are highlighted to suggest that such changes may already be taking place.

Vector immunity

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Many invertebrates, but particularly insects, act as vectors of debilitating diseases in the developing world. These parasites are not simply transported from human to human by their vectors but interact intimately with their invertebrate hosts, usually undergoing significant biochemical and molecular modifications in order to survive, differentiate and multiply in their vectors. The present review focuses on the immune system of the vectors and the steps taken by invading parasites to avoid or manipulate these host defence processes. Important factors considered include the parasite life cycle and the immune potentials of the different compartments encountered in the vector. The vector compartments and barriers to infection described include physicochemical barriers, the gut barrier, the haemocoel and the salivary glands. Vector immune processes interacting with parasites are the cellular defence reactions, such as phagocytosis and encapsulation responses, while humoral factors described are the prophenoloxidase system, pattern recognition receptors, non-self recognition processes and killing factors, including reactive oxygen and nitrogen intermediates. It is hoped that the identification of key vector immune factors determining the development of parasites will assist the quest for infection blocking genes or even the development of transmission-blocking vaccines.

Transmission of plant viruses by nematodes

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Viruses belonging to two genera are transmitted between plants by soil-inhabiting, plant parasitic nematodes. Trichodorid nematodes (*Trichodorus* spp., *Paratrichodorus* spp.) transmit the tobraviruses, whereas, longidorid nematodes (*Xiphinema* spp., *Longidorus* spp.) transmit the nepoviruses. Both groups of nematodes are ectoparasitic, remaining outside the root and using needle-like stylets to pierce epidermal cells during feeding. As the plant cell contents are ingested by the nematode, virus particles become adsorbed to the nematode oesophageal cuticle where they are retained before being released into further plants during subsequent feeding episodes. This presentation will give an overview of plant virus transmission by nematodes, including an introduction to tobraviruses and nepoviruses and the diseases they cause, as well as a discussion of recent work to identify the specific viral proteins that are involved in the transmission process.

Wolbachia host–symbiont interactions

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Wolbachia are obligate intracellular bacteria that infect a wide range of invertebrate hosts. As a genus they are most closely related to the arthropod-transmitted bacteria *Ehrlicha* within the Rickettsiacae. *Wolbachia* are widespread throughout insects and also infect crustaceans, mites and filarial nematodes. *Wolbachia* have evolved a diversity of associations with their hosts, ranging from being pathogens and reproductive parasites in arthropods to mutualists in filarial nematodes. A common target of the interaction of *Wolbachia* is the host's reproductive system, which is manipulated to enhance the maternally inherited spread of the symbiont throughout populations by inducing mating incompatibility,

parthenogenesis, feminization and male-killing. The mutualistic dependence of filarial nematodes on *Wolbachia* has been used in a new approach to the treatment of filariasis. Treatment with antibiotics leads to an arrest of larval and embryonic development and survival and can also lead to the eventual death of adult worms. Insect *Wolbachia* have also been proposed as a tool for the control of pests and vectors of disease. The diversity of association and phenotype in *Wolbachia* provides an excellent opportunity to study the nature, mechanisms and evolution of these different host–symbiont interactions.

Pathogenic strategies of *Anaplasma phagocytophilum*, a unique bacterium that colonizes neutrophils

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Anaplasma phagocytophilum is a causative agent of human granulocytic ehrlichiosis, an emerging tick-borne zoonosis in the United States and Europe. This tick-transmitted, obligate intracellular bacterium is unique in that it colonizes polymorphonuclear leukocytes (neutrophils). Neutrophils are primary players in innate immunity. *A. phagocytophilum's* tropism for neutrophils is paradoxical and indicates that it utilizes strategies for circumventing and/ or subverting the bactericidal activities of its host cell. This review summarizes current knowledge regarding the mechanisms *A. phagocytophilum* uses for immune evasion, host adaptation, neutrophil adhesion, intra-neutrophil survival, and its dissemination to naïve cells.

Plague in fleas

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The arthropod-borne route of transmission of *Yersinia pestis*, the agent of plague, is an evolutionarily recent adaptation that distinguishes it from closely related enteric pathogens. To produce a transmissible infection in its flea vector, *Y. pestis* colonizes the digestive tract and eventually blocks the foregut by growing in the form of a dense aggregate that is enclosed within an extracellular matrix, a phenotype that fits the definition of a bacterial biofilm. *Y. pestis* genes that are specifically required to infect and block fleas, termed transmission factors, have been identified using the rat flea *Xenopsylla cheopis* as a model organism, and some of the genetic steps leading to the rapid evolutionary transition to flea-borne transmission can now be surmised. These steps included the acquisition of new plasmid-borne genes by horizontal transfer and the application of pre-existing chromosomal genes to new functions that are specifically required in the insect vector. Perhaps reflective of recent adaptation, *Y. pestis* transmission by fleas is inefficient, which likely imposed selective pressure favoring the emergence of highly virulent clones.

Transgenic malaria

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The ability to genetically engineer mosquito species that transmit the *Plasmodium* pathogen has enabled genetic-based strategies aimed at preventing pathogen transmission through the mosquito to be investigated. These studies, reviewed here, illustrate that a significant reduction in pathogen numbers within the transgenic female mosquito can be obtained, with a resulting decrease in the transmission of *Plasmodium* to uninfected vertebrate hosts. The significant challenges that still remain are the complete elimination of *Plasmodium* transmission through mosquitoes, the identification of genetic drive mechanisms to spread beneficial transgenes through mosquito populations in the field, and the public acceptance of transgenic mosquito strains. Here we review progress in all three of these areas of modern transgenic mosquito research.

Vaccines targeting vectors

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Haematophagous arthropods induce immune responses in the hosts on which they feed but have an array of immunomodulatory factors to modulate mechanisms that are harmful. Blood feeding by insects and ticks forms the route of transmission of many infections of medical and veterinary importance and, vaccination directed against the arthropod can thus serve the dual purpose of interfering with fecundity and survival of the arthropod, and impairing its ability to serve as a vector of disease.

Commercially available anti-tick vaccines are based on identified mid-gut glycoproteins. These normally concealed antigens induce antibodies that damage mid-gut cells and, as a result, the ticks are less able to engorge blood, and their weight and reproductive capacity are reduced. A similar approach to vaccination against the mosquito vectors of malaria parasites, though at present less well characterized, reduces parasite development on the mid-gut of the mosquito.

An alternative approach is to vaccinate against salivary components. Sandfly saliva proteins promote transmission of leishmanial parasites, and vaccination against selected salivary proteins impairs their vasodilatory and immunomodulatory effects, and reduces the infectivity of *Leishmania* metacylic stages.

The approaches to vaccination mostly involve use of crude antigens and, if these are to be exploited, the vaccine candidates must be better defined so that use can be made of new vaccine technologies.

Cells & Cell Surfaces Group session

Surface mediators

Bacterial resistance to innate immunity

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Abstract not received

The staphylococcal cell envelope and its role in infections

Christopher Weidenmaier[1], Sascha Kristian[2], Petra Staubitz[1], Iris Fedke[1], Manuela Dürr[1], John F. Kokai-Kun[3], Regine Landmann[2], L. Vincent Collins[4] & **Andreas Peschel**[1]

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Staphylococcus aureus is a major human pathogen in hospital- and community-acquired infections, causing wound infections, bacteremia, and sepsis with a high mortality rate. The staphylococcal cell envelope contains teichoic acids (wall teichoic acids, WTA; lipoteichoic acids, LTA), complex surface-exposed polymers, whose potential role in infections has remained elusive because of the lack of WTA or LTA mutants. We generated the first defined WTA-deficient mutant and demonstrated that WTA is crucial for several aspects of *S. aureus* virulence.

Colonization of the anterior nares by *S. aureus* in ~37% of the population is a major risk factor for severe infections. WTA was shown to be essential for nasal colonization and to mediate specific interaction with human nasal epithelial cells. Epithelial cells and granulocytes produce defensins and other antimicrobial peptides to prevent bacterial colonization and infection. Modification of teichoic acids with D-alanine was shown to protect *S. aureus* against defensins and to represent a critical virulence determinant. Several pathogenic bacteria produce unusual L-lysine-modified phospholipids. We showed that phospholipid lysinylation has a similar impact as teichoic acid alanylation and contributes to defensin resistance by reducing the anionic bacterial surface charge.

Taken together, our studies demonstrate that non-proteinaceous cell wall components deserve more attention as mediators of bacterial colonization, immune escape, and inflammation. Moreover, the highly conserved biosynthetic enzymes of cell wall polymers represent interesting new targets for novel antiinfective strategies.

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Role of NOD2 in innate immunity and inflammatory disease

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NODs are members of a family of cytosolic proteins with homology to plant disease resistance (R) gene products. R proteins control the defense response of plant cells to invading pathogens. We have identified 24 NOD genes in the human genome. NODs including NOD1 and NOD2 contain variable N-terminal effector domains, a centrally located nucleotide-binding oligomerization domain (NOD) and C-terminal leucine-rich repeats (LRRs). Mutation in three NOD proteins (NOD2, Cryopyrin and CIITA) are associated with inflammatory disease or immunodeficiency. NOD1 and NOD2 recognize conserved but distinct structural motifs in bacterial peptidoglycan through their LRRs and induce the activation of NF-κB. Activation of NF-κB through NOD1 and NOD2 is mediated through RICK, a serine/threonine kinase that interacts with the IκB kinase (IKK) complex. Mutation and genetic variation in the LRRs of NOD2 are associated with susceptibility to Crohn's disease and Blau syndrome. NOD2 variants associated with Crohn's disease are deficient in NF-κB activation induced by bacterial peptidoglycan and specifically muramyl dipeptide. In contrast, NOD2 mutations associated with Blau syndrome function as activating mutations. Systematic mutational analyses revealed a general mechanism for recognition of pathogens by the LRRs of NOD2. Our data indicate that NODs including NOD1 and NOD2 function as intracellular receptors for specific microbial components leading to the activation of a cellular response against the pathogen. NOD2 mediates the host response to bacterial muropeptides, an activity that is important for protection against Crohn's disease.

Mechanism of meningococcal type IV pilus mediated adhesion

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Neisseria meningitidis is a commensal of the human nasopharynx which in some circumstances can invade the bloodstrean, cross the blood brain barrier and invade the meninges. The bacteria enter the central nervous system following a direct interaction with the luminal side of the cerebral endothelium, which constitutes the blood–brain barrier. To breach the barriers protecting the brain, *N.meningitidis* must cross a monolayer of tight junction-expressing endothelial or epithelial cells. Meningococcal type IV pili have been identified as being essential for the initial adhesion.and invasion by inducing the formation of microvilli like structures on the apical surface of the endothelial cells. However following the initital adhesion step pili retract, bacteria become non piliated and are intimately associated with the cells. This induction of pilus retraction during bacterial adhesion raise two kinds of issue (i) what are the mechanism responsible for the induction of pilus retraction during the host cell interaction, and (ii) what are the molecular mechanisms responsible for type IV pilus mediated adhesion considering that pili are dispensable at the intimate adhesion step. In order to address these questions we have recently used a genome wide collection of mutants which has been screened for adhesion and piliation.

MALP-2, a toll-like receptor agonist from mycoplasmas: structural requirements and biological activities

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MALP-2 (macrophage activating lipopeptide of 2 kDa mol. mass) was originally isolated from *mycoplasma fermentans* (Muhlradt, 1997). It is a cleavage product of the lipoprotein MALP-404 (Calcutt, 1999). MALP-2 is now synthetically available. *In vitro* it stimulates the release of various mediators, such as cytokines and chemokines, and modulates the cell surface. The spectrum of the mediators varies, and depends on the respective potential of the target cell, as does the modulated expression of cell surface molecules. Among the target cells are: monocytes/macrophages, dendritic cells, B cells and

fibroblasts. One prerequisite for MALP-2 function on the target cell side is the functional expression of toll-like receptors 2 and 6 (Takeuchi, 2001). Biological activity and receptor requirement of MALP-2 is dependent on (i) the stereochemistry of the lipid moiety, (ii) the presene of two ester-bound long chain fatty acids, (iii) a free amino terminal, (iv) an intact thioether group bridging lipid and peptide moiety (Morr, 2002). MALP-2 shows distinct *in vivo* activities and can be used as an adjuvant (Rharbaoui, 2002), to induce partial endotoxin tolerance (Deiters, 2003), and to accelerate wound healing (Deiters, 2002). I shall try to discuss these seemingly independent activites with respect to known signal pathways and to relate them to reported effects of mycoplasma infections.

Modulation of immune responses by *Bordetella pertussis* virulence factors

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Abstract not received

Changes in host responses mediated by the meningococcus

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The interaction of *Neisseria meningitidis* with the meninges that surround and protect the brain is a pivotal event in the progression of bacterial meningitis. An *in vitro* cell culture model of the human meninges was established, which permitted studies of the ligands present on the meningococcal surface that mediated pathogen–host meningeal cell interactions. One consequence of these interactions was the secretion of a specific subset of pro-inflammatory, chemoattractant and growth factor-related cytokines by meningeal cells; demonstrating that these cells are not inert but active participants in the inflammatory response and that they play a major role in innate host defence during meningococcal meningitis. Moreover, a complex relationship between expression of meningococcal surface ligands and induction of these inflammatory mediators was observed. Recently, we have investigated the interactions of other major pathogens causing meningitis – *Haemophilus influenzae, Streptococcus pneumoniae* and *Escherichia coli* K1 – and compared and contrasted the biological effects with those induced by meningococci. These studies demonstrated that the interactions *in vitro* between bacteria causing meningitis and human meningeal cells were notably different, with regards to bacterial adhesion and invasion and the ability to induce host cell death and modulate the innate inflammatory response.

Epithelial responses to *Helicobacter pylori* and implications for gastric cancer

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The interaction of *H. pylori* with epithelial cells and associated changes in epithelial gene expression and function are critical in generation of the host's inflammatory response and the development of chronic gastritis. The risk of developing atrophic gastritis and gastric cancer with *H. pylori* infection is related to both strain variation and polymorphisms in host inflammatory response genes. Strains with the *cag* pathogenicity island (PAI) are associated with increased risk of atrophic gastritis and intestinal type gastric cancer. The *cag* PAI, which encodes a type IV secretory system, is present in 50–60% of clinical isolates. Strains with the *cag* PAI translocate the CagA protein into gastric epithelial cells where it is phosphorylated by host cell tyrosine kinases and induces a range of cytoskeletal changes. Translocated CagA also alters epithelial barrier function by disrupting apical junctional complexes. Strains with the *cag* PAI activate NFκB, mitogen activated protein kinase (MAPK) pathways and upregulate proinflammatory genes such as IL-8 in gastric epithelial cells. The epithelial signalling response accounts for the enhanced neutrophilic infiltration and increased risk of developing gastric cancer. Two other bacterial virulence factors also associated with increased risk of carcinogenesis are the vacuolating cytotoxin encoded by *vacA* and the adhesin *babA2*.

The cellular and molecular pathways by which infection and chronic inflammation promotes transformation remain to be resolved. Gastric epithelial cell proliferation is increased in *H. pylori* infection. *H. pylori* transactivates the epidermal growth factor receptor (EGFR) in gastric epithelial cells and this is dependent on extracellular transmembrane metalloprotease cleavage of pro-heparin binding epidermal growth factor (proHB-EGF) and signalling by mature HB-EGF. Activation of this signalling pathway will promote gastric epithelial cell proliferation and decrease apoptosis. Overexpression of key elements of the signalling cascade such as HB-EGF and ADAM membrane metalloproteases in patients with gastric cancer or atrophic gastritis suggests the EGFR autocrine/paracrine signalling pathway induced by *H. pylori* is of pathophysiological relevance.

Clinical Microbiology Group / British Infection Society joint session

Infections in the global village

Pathogenesis of malaria

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The symptoms of malaria range from a mild, febrile syndrome accompanied by nausea and headache through to severe anaemia, metabolic acidosis, cerebral malaria and multi-organ disease, which may present as renal failure or pulmonary oedema. Whereas some symptoms, such as anaemia, are primarily caused directly by the parasite, many of the clinical features are related to the immune response – characterised by high levels of circulating inflammatory cytokines. Parasite products, containing glycolipids or phospholipoproteins, can directly induce macrophages to produce TNF-α/LT-α, IL-1 and IL-6 (all of which are endogenous pyrogens) and this response is enhanced by IFN-γ, which may come from NK cells, $\gamma\delta$ T cells or malaria-specific $\alpha\beta$ T cells. Furthermore, the ability of some species of *Plasmodium* to bind to vascular endothelium seems to be a fundamental component of life-threatening pathology. People living in malaria endemic areas eventually develop clinically protective immunity, such that they are able to control the levels of parasites in their peripheral blood and no longer develop severe symptoms of disease, but sterilising immunity is rare and most clinically immune individuals will experience periodic, asymptomatic infection. The effector mechanisms of protective immunity are not fully understood but include both anti-parasitic mechanisms and development of responses to minimise pathology.

Progress with malaria vaccines

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A malaria vaccine is needed urgently to prevent the deaths of more than 2,000 African children each day. Our vaccine development program is aimed at producing such a vaccine. However malaria is also an excellent model for other infectious diseases in which a T cell response may be protective, since infectious challenge to test vaccine efficacy may be carried out in humans as well as in animal models. Results from clinical trials of a malaria vaccine designed to induce T cell responses against the liver stage of *Plasmodium falciparum* will be presented. The same test antigen, consisting of a multiple epitope string containing epitopes derived from six liver-stage antigens, fused to the complete coding sequence of TRAP, was used in three different vaccine delivery systems: DNA, Modified Vaccinia virus Ankara (MVA) and an attenuated fowlpox (FP9). These vaccines have now been tested in clinical trials, both on their own and in heterologous prime-boost regimes. As expected, the prime-boost regimes resulted in considerably greater T cell responses and protective efficacy than the use of any of the delivery systems alone.

Rapid diagnosis of tropical infections

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Abstract not received

TB and HIV: an unholy alliance

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TB and HIV are common co-pathogens and they exert synergy at cell, molecular and clinical level through the common cell which they infect, namely the macrophage. Infection of macrophages by Mycobacterium tuberculosis (mTB) activates NF-κB and induces a cascade of cytokine release including TNFα and a range of chemokines. HIV transcription in macrophages is largely regulated by NF-κB through two repeat motifs in LTR and co-infection with mTB drives HIV transcription both through NF-κB and in an autocrine fashion through TNFα. Furthermore cognate interaction of primed activated T cells with macrophages containing mTB during granuloma formation drives further HIV transcription through adhesion molecule engagement. The principal adhesion molecules involved are ICAM and LFA-1.

Treating HIV negative patients infected with mTB with anti-TB chemotherapy restores weight, haemoglobin and well-being whilst reducing plasma CRP. Paradoxically in such patients plasma HIV copy number does not fall but the phenotype of the viral envelope alters considerably to reflect the reduced level of activation of the parent cell. In such patients plasma levels of TNFα remain elevated in the face of TB treatment and the raised level of this pro-inflammatory cytokine may be responsible for unabated HIV transcription.

The future of medical microbiology services

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The delivery of Medical Microbiology Services to the NHS in England and Wales is undergoing major change with the creation of the Health Protection Agency, the transfer of responsibilities for most former PHLS Laboratories in England to their NHS Trusts, the establishment of the National Public Health Service for Wales, the pathology modernisation programme and the appointment of an Inspector of Microbiology, initially in the Department of Health but due to transfer to the Commission for Health Audit and Inspection in 2005/06. Because of the nature of infection, all microbiology laboratories need to contribute to both clinical care of individual patients and health protection/public health functions. The aim is an integrated and cohesive microbiology and virology service delivered by qualified and trained staff in NHS, HPA, university and private laboratories that are working in partnership, accredited, and quality assured through inspection. Laboratories serving the NHS should be working to agreed standard operating procedures and algorithms that support clinical medicine and contribute to surveillance, outbreak investigation, look back exercises and the immediate local response to deliberate release or natural incidents. The role of the Inspector is to ensure that this happens, working with microbiology and service recipient stake holders to develop a networked, consistent service, with agreed standards that are reflected in the inspection process.

Novel mucosal vaccines against enteric infections

D. Lewis

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Abstract not received

Role of man as the reservoir for tick-borne relapsing fever in Tanzania

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Tick-borne relapsing fever, caused by the spirochaete *Borrelia duttonii*, is endemic in Tanzania. The tick vector, *Ornithodoros moubata*, which frequently infests traditional style 'Tembe' houses, transmits the disease. We have assessed the incidence of borrelial carriage by PCR in asymptomatic villagers to address whether man could serve as the reservoir for this disease.

We used this PCR on a cohort of 163 Tanzanian villagers from four separate villages within the Dodoma Rural District, Central Tanzania: Mvumi Makulu; Iringa Mvumi; Ikombilinga and Makang'wa. DNA was extracted using the MagnaPure system (Roche) from whole blood. PCR amplicons were visualised using Microplate Diagonal Gel Electrophoresis (MADGE). PCR results demonstrated that 58% (163) of the cohort tested gave a PCR positive result unlike the 11% reported by Kisinza *et al.*[1] from a smaller collection of samples in the same geographical area. These researchers reported the presence of a novel *Borrelia* spp. In order to characterise the PCR amplicon, we subjected these to Restriction Enzyme (RE) digestion using S*sp*I. With this enzyme classical *B. duttonii* yields one control band (123bp) and a further band (147bp). While the new *Borrelia* fails to show the 147bp band. Of those tested 68.1% (111) gave the expected profile for *B. duttonii*, while 14.7% (24) were compatible with the new *Borrelia spp* described[1]. However 5.5% (9) produced a unique digestion profile with a further 11.7% (19) lacking a digestion site. Failure of the RE to cleave these latter two categories suggests variation at the sequence level that may be indicative of a further as yet unclassified *Borrelia* species. This will be resolved using sequencing data.

Furthermore, clustering of infected individuals was demonstrated within occupants of the same huts. RE profiles correlated with families suggesting that they were infected with the same strain. In conclusion, we have demonstrated the presence of *Borrelia* in 58% of villagers, suggesting that man may indeed be the reservoir for this disease. Whether all of the borreliae detected have pathogenic potential remains to be ascertained. Our finding of *Borrelia* DNA in 58% of asymptomatic villagers would suggest that indeed man is a major reservoir for tick-borne relapsing fever in Tanzania.

Reference: Kisinza, W.N., McCall, P.J., Mitani, H., Talbert, A., Fukunaga, M. (2003). A newly identified tick-borne *Borrelia* species and relapsing fever in Tanzania. *Lancet* **362**, 1283–1284.

Importation of rabies into the United Kingdom

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Rabies Research and Diagnostic Group, [WHO Collaborating Centre for the Characterisation of Rabies and Rabies-Related Viruses], Veterinary Laboratories Agency (Weybridge), Surrey

Unrestricted travel to rabies-endemic countries is increasing the risk that a case of human rabies may be imported into the UK. In 2001, the UK had two confirmed human rabies cases, imported from the Philippines and Nigeria. In the Philippines case, molecular diagnostic techniques confirmed the presence of rabies virus from ante-mortem specimens taken within 36 hours of sample submission. Subsequent phylogenetic analysis demonstrated that the virus was closely related to that of canine variants of classical rabies virus (genotype 1) circulating in the Philippines. In the Nigerian case, the diagnosis of rabies was confirmed on post-mortem tissue. Phylogenetic analysis of two genomic segments of this isolate confirmed that it was a classical rabies virus strain of the Africa 2 sub-group.

In the UK, a strain of European Bat Lyssavirus (EBLV) type-2 (genotype 6) has also been isolated from insectivorous bats. Two isolates were confirmed in Daubenton's bats (*Myotis daubentonii*), in Sussex (1996) and Lancashire (2002). The virus isolated from the Daubenton's bat in Sussex (1996) was thought to have migrated from mainland Europe and was not considered at the time to have been from an indigenous British bat. Phylogenetic comparison of these two isolates with published sequences from genotype 6 suggests links to bat strains of EBLV-2 isolated from Northern Europe. A third EBLV-2 isolate was responsible for the death of a bat handler in Scotland in November 2002. Surveillance is currently underway to fully assess the true prevalence of EBLVs in British bat populations and to more fully understand whether EBLVs are enzootic in the UK or are a consequence of periodic bat migration.

Adherence patterns of putative enteroaggregative *E. coli* from patients with traveller's diarrhoea

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Enteroaggregative *Escherichia coli* (EAEC) are emerging as a significant cause of severe, persistent diarrhoea in both adults and children. Strains of EAEC are defined by their distinctive 'stacked brick' pattern of aggregative adherence (AA) to epithelial cells *in vitro*. The aim of this study was to evaluate the actual adherence patterns of isolates hybridising with the widely used DNA probe for EAEC, known as CVD432 or AA. *E. coli* isolates (n=440) were collected from consecutive stool samples from 110 diarrhoea patients with recent histories of foreign travel, and screened for their ability to hybridise with the CVD432 probe. Hybridising strains were tested for aggregative adherence to HEp-2 cells. Seven percent (31) of the isolates were probe-positive. Of these, 48.4% (15 isolates) adhered to HEp-2 cells, with varying degrees of aggregation, and 19.4% (6 isolates) were non-adherent. The remaining 32.2% (10 isolates) gave an unusual pattern of loose, highly localised aggregation, present on <1/20 of the HEp-2 cells. The positive predictive value for the CVD432 probe for aggregative adherent *E. coli* was just 48.4% in this study, emphasising the need to develop better probes / tests for identifying these pathogens in the diagnostic setting. The significance of the newly described adherence pattern in relation to disease remains to be elucidated.

Staphylococcus aureus with reduced vancomycin susceptibility have evolved within all five pandemic MRSA lineages

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Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) with reduced vancomycin susceptibility (SARVS) which include VISA (vancomycin intermediately-susceptible *S. aureus*) and heterogeneous VISA (hVISA) have evolved in many countries due to increases in vancomycin use. It is currently unclear whether SARVS isolates are evolving regularly in different genetic backgrounds or if one clone alone has developed resistance, as early results suggest.

Methods: 102 MRSA isolates from 9 countries were studied: 10 VISA (MIC ≥ 8 µg/ml) and 92 hVISA (confirmed by population analysis profiling). Multilocus sequence typing (MLST), characterization of the mobile genetic element carrying the structural gene (*mecA*) for

methicillin resistance – SCC*mec* (staphylococcal chromosomal cassette *mec*) and *agr* typing were used to examine the strain genetic background.

Findings: 9 MLST genotypes were found, corresponding to isolates within all 5 pandemic MRSA lineages. 14/102 isolates evolved from MRSA from 3 lineages first described in the 1990s. The majority (88) of SARVS evolved from 2 early MRSA lineages that have greater geographical prevalence. There appears to be no link between *agr* type and SARVS phenotype, with *agr* types distributed across clonal complexes as expected.

Conclusion: The SARVS phenotype appears to have risen in the major pandemic MRSA lineages, perhaps due to their global overrepresentation, and there also seems to be no correlation between the *agr* type and SARVS phenotype for an isolate.

Tropical sexually transmitted diseases

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The last decade has significant advances in our understanding of chancroid, donovanosis and lymphogranuloma venereum. Of these infections, chancroid remains must the most common, but there are suggestions that disease it is being displaced by HSV in areas of high HIV prevalence. Research into the basic science of *H. ducreyi* continues apace leading to a much more complete picture of pathogenesis. The successful culture of Gram negative bacteria from donovanosis lesions after a hiatus of over 30 years has led to the development of new diagnostic tools and a contentious reassignment of the causative organism to the genus *Klebsiella*. A donovanosis eradication programme in Australia, utilizing azithromycin for treatment, has made excellent progress. Lymphogranuloma venereum remains the most neglected of these three diseases but even here recent research from South Africa using more sensitive and specific diagnostic tools has demonstrated that the primary lesion of this disease has a considerably wider range of appearances than hitherto realized. It has been suggested that a number of tropical infections (most notably schistosomiasis) which are not sexually transmitted but which lead to the development of genital lesions, may potentiate the the transmission of HIV or accelerate HPV-oncogenesis.

Sport and infection

Nick J. Beeching

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Participation in sports can predispose towards a variety of mundane and exotic infections. Water contact has been associated with cercarial dermatitis in bathers, schistosomiasis in windsurfers and kayakers, leptospirosis in triathletes, hantavirus infections in white water rafters and otitis externa in surfers. Ingestion of contaminated water has caused outbreaks of hepatitis A, giardiasis and cryptosporidiosis in bathers. Ground contact in sport has resulted in a variety of forms of cutaneous and visceral larva migrans. Close contact sports result in person to person transmission of herpes infections (herpes gladiatorum) and dermatophyte infections, and blood borne viruses such as hepatitis B have been spread among college football players and orienteers. Athletes who venture into new environments have acquired legionellosis on golf courses, tick typhus, cutaneous leishmaniasis and cutaneos myiasis in different exotic settings. All travelling sports participants are at risk from traveller's diarrhoea, respiratory and sexually transmitted infections.

This talk will illustrate sport related outbreaks of infection and review the limited evidence base for prevention of specific infections in sports settings, including discussion about regulations for control of blood borne viruses in contact sports. The wider issues of community infection control at large sporting events such as the Olympics will also be addressed.

Impact of migration on the epidemiology of tuberculosis: insights from molecular studies

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The advent of molecular methods for typing isolates of *Mycobacterium tuberculosis* made it possible for the first time to use strain typing as a tool for investigating possible outbreaks of tuberculosis. The discriminatory power of these methods is such that the identification of identical isolates can be taken as evidence of an epidemiological connection, even in the absence of any direct information on the existence or nature of such a link. Using lower levels of similarity, it is possible to divide *M. tuberculosis* isolates into a number of 'families' which provide evidence of longer-range spread of the bacterium. Predominant amongst these is the Beijing family, which was first identified in isolates from China and is the major family of strains from many parts of Eastern Asia. This family has subsequently been identified in many other parts of the world, and has been responsible for many outbreaks of drug-resistant tuberculosis. This paper will discuss the world-wide spread of the Beijing family, and other families of *M. tuberculosis* strains and the factors that influence this dissemination.

Praziquantel resistance in schistosomes

Michael J. Doenhoff

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Praziquantel (PZQ) is now virtually the only drug used routinely to treat schistosomiasis. A single dose of ~40mg/kg is effective against all 3 main species of schistosome infecting humans and it can be administered orally and is very safe. Its rate of use is likely to increase markedly because of dramatic price reductions in the last few years, and a major initiative aimed at reducing schistosome-induced morbidity in school children has begun in several countries in Africa. The anticipated greatly increased rate of usage of this drug has generated concern about drug-resistance.

In the early 1990s PZQ gave poor cure rates when it was used to treat an outbreak of schistosomiasis mansoni of 'epidemic' proportions in Senegal. There has been considerable argument whether the poor efficacy in Senegal was due to drug-resistance or a consequence of the unusually high levels of infection intensity and transmission in that area.

In the meantime it has been shown that *Schistosoma mansoni* can be selectively bred in the laboratory to become partially resistant to PZQ. Furthermore, some isolates of *S. mansoni* taken from patients who were not cured after several treatments have been shown to have higher PZQ ED_{50}s than control isolates which had not been exposed to the drug. It can thus be concluded that schistosomes have the capacity to develop a partial resistance to PZQ, but the implications of this for the future use of the drug are not yet clear.

Currently very little is known about the mode of action of PZQ and nothing about mechanisms of resistance to it. Work is underway to try and fill these serious gaps in our knowledge.

Flaviviruses: here there and everywhere

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Following the unexpected appearance of the flavivirus West Nile virus (WNV) in North America in August 1999 and its subsequent widespread dispersal in the Americas, arbovirology in the USA received a major injection of dollars and scientific interest. However, other arthropod-borne viruses (arboviruses) such as dengue, yellow fever, Japanese encephalitis, St Louis encephalitis and tick-borne encephalitis are all equally, if not more important pathogens of humans and animals and each has an interesting story to tell. This presentation will examine the phylogenetic relationships of these and other related flaviviruses and show how their evolution, epidemiology, and dispersal are dependent on the arthropods and vertebrate hosts that they infect, as well as the climate, and the influence of human activities such as transportation, urbanization, air/sea travel and farming.

Cysticercosis

J. Friedland

Imperial College London

Abstract not received

Clinical Virology Group / Association of National Health Occupational Physicians joint session

Workplace-associated virus infections

Overview and where we're going next?

F. Ncube

Health Protection Agency, London

Abstract not received

Risks to patients of acquisition of HBV, HCV and HIV from infected health care workers

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Blood-borne viruses (BBV, principally HBV, HCV and HIV) can be transmitted from infected patients to health-care workers (HCWs) and also from infected HCWs to patients.

In an attempt to reduce the risk of HCW-to-patient transmission of HBV, HCV, and HIV, the Department of Health (DoH) have brought in various guidelines concerning the fitness to practice of HCWs infected with these viruses.[1,2,3] As a result of these, HBV-infected HCWs who perform exposure prone procedures (EPPs) must be shown to have a viral load $<10^3$ copies/ml; new entrants to specialties which require the performance of EPPs are required to be tested for HCV infection, and HCV viraemic individuals will be banned from EPPs. HCWs known to be infected with HIV are not allowed to perform EPPs. There are proposals to extend these regulations to all new entrants into the NHS, including students.[3]

Devising appropriate strategies to protect patients whilst also being fair to infected HCWs has proved difficult. This talk will review the evidence of BBV transmission from HCWs to patients, and the background to the new regulations, and identify and discuss the controversies they have inevitably generated.

References: [1]Hepatitis B Infected Health Care Workers. Health Service Circular (HSC) 2000 /020; [2]Hepatitis C Infected Health Care Workers. Health Service Circular (HSC) 2002 /010; [3]Health Clearance for Serious Communicable Diseases: New Health Care Workers (draft guidance for consultation January 2003)

An audit of immunisation practice

Peter Verow

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The Association of NHS Occupational Health Physicians (ANHOPS) has long been aware of variations in immunisation practice between NHS Occupational Health services. It was believed that this was due to a number of factors – lack of specific OH guidance, variation in interpretation of relevant immunisation guidance, and a lack of resources to implement guidelines fully.

The recent guidance on varicella is a case in question. The guidance comes with no resources but a recommendation to immunise all non immune clinical health care workers. Such a programme will be expensive and whilst most occupational health services may want to proceed with such a programme they may not have the budgets to do so. They may have to prioritise testing and vaccination for clinical staff within maternity, paediatric and immuno-compromised areas. Whether they will be able to find the resources to immunise other areas of lower risk may be more doubtful.

In 1998 Dr Ian Blair (CCDC Sandwell) and myself, Dr Peter Verow (Consultant OHP Sandwell) felt that we should try to identify the variation in immunisation practice between the various occupational health services. The aim was then to use this information to suport the need for more specific guidance within the 'Green Book' for the immunisation of health care workers. The results verified the need for this and the Deptartment of Health did begin to produce such a document however nothing has ever been finalised. A further audit was undertaken in January 2004 to see if the situation had changed and these results will be presented at this meeting.

Varicella

Elizabeth Miller

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Chicken pox is generally perceived as a mild self-limiting disease in countries where the majority of cases occur in children. In immunocompetant children, complications are rare with less than two deaths per 100,000 cases in children age 1 to 14 years. In England and Wales over the period 1991 to 1995 there were on average 4 deaths a year in children under 15 years of age; three of these deaths would have been in previously normal, immunocompetant children. Children under 15 years of age comprise around half of all hospital admissions for chicken pox, the annual total for which in England and Wales is between 2000 to 3000. In contrast to children, chicken pox in adults is more frequently associated with complications and death. Clinically apparent varicella pneumonia occurs in approximately 1 in 400 adult cases and is particularly severe in smokers. Other consequences of infection in adults arise when varicella occurs during pregnancy. Maternal infection in the first 20 weeks carries a small risk (approximately 1 to 2%) of congenital varicella syndrome characterised by limb hypoplasia, skin scarring and neurological damage. Varicella later in pregnancy can give rise to premature delivery and, if onset is before term, severe varicella in the neonate. A live attenuated vaccine against varicella was developed in Japan in 1970 and has recently been licensed for use in immunocompetant children in the US. The case for introducing varicella vaccine into the UK immunisation programme will be discussed in the light of current evidence about its safety and efficacy, and compatibility with other vaccines. The long term implications of mass immunisation against chickenpox on the epidemiology of varicella and herpes zoster will be considered.

Measles immunity and screening in health care workers

E. Ziegler

Occupational Health Dept, Southampton University Hospital Trust

Health care workers (HCW) are at greater risk of measles than the general population. With the decrease in MMR immunisation rates in the general population there are likely to be more outbreaks of measles and more hospital admissions. This presentation will discuss both an original research study undertaken by the author and colleagues (Cambridge study) and related research from other sources. Conclusions will be drawn about the need for guidance in this area of practice.

The Cambridge study determined the prevalence of measles non-immunity in a group of HCW, identified the most appropriate algorithm to identify these individuals, and explored current practice in UK NHS trusts.

The cross sectional study of HCW (218) on 11 medical wards, using a self administered questionnaire and laboratory assessment of measles immunity was undertaken together with a postal survey of Association of National Health Occupational Physicians (ANHOPS) members.

3.3% HCW were non-immune to measles with over 50% of them born before 1970. Oral fluid testing and history of measles disease or vaccination are unreliable methods to identify non-immune individuals. Of a sample of 80 NHS occupational health departments, 31% inquired about measles immunity and of these 80% relied on history to determine immunity. Serum testing is the most reliable method for identification of measles susceptible individuals.

Cost, ethical and legislative issues together with changing demography of work force are important when considering guidelines that need correct implementation to ensure uniform standards across the NHS.

Influenza: health care worker vaccination

Iain Stephenson

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Influenza is a major cause of morbidity and mortality. Vaccination of HCWs has been suggested to reduce nosocomial influenza transmission and staff absenteeism. Although vaccine is highly efficacious, uptake rates are poor. Active intervention can increase uptake; associated factors include occupation, previous influenza vaccination and belief that vaccine is effective. Negative factors include side-effects and poor efficacy. HCWs are implicated as sources of hospital outbreak in adult and paediatric settings. Upto 23% HCWs have serological evidence of influenza infection and often attend work despite illnesses. Analysis of influenza-like illnesses (ILI) among elderly residents in 149 Japanese nursing homes during an H3N2 epidemic found resident and staff vaccination rates both independently associated with significant reductions in ILI among residents (OR 0.41, 95% CI 0.36–0.56 where staff vaccination rates exceeded 20%). A Finnish placebo-controlled double-blind trial found a significant 28% reduction in absenteeism related to respiratory infections. However, two other placebo-controlled studies totalling 443 HCWs found no differences. Although influenza vaccine is well tolerated in studies, many HCWs perceive vaccine-related effects. In Vancouver, 6% recipients reported missing work because of vaccine-related symptoms. In Leicester, 38 lost working days were attributed to vaccine-related effects amounting to 0.46 days per vaccine. A significant number of HCWs 'do not want' influenza vaccination. Some may be persuaded by active promotion, but substantial numbers will likely remain unvaccinated. The current strategy of promoting vaccine availability may not translate into increased uptake and resources may be required to deliver improved access and delivery of vaccine. Large-scale randomised studies of influenza vaccination of HCWs need to address health-economic and cost-effectiveness issues.

Workplace associated virus infections – Norovirus virology

Jim Gray

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Enteric viruses can be categorised into:

1. Viruses causing gastroenteritis: adenoviruses (types 40 and 41), astroviruses, noroviruses, rotaviruses and sapoviruses.

2. Viruses associated with systemic infections: enteroviruses (polioviruses, coxsackie viruses and echoviruses) and parechoviruses.

3. Viruses associated with hepatitis: hepatitis A virus (HAV) and hepatitis E virus (HEV)

Noroviruses are the major cause of viral gastroenteritis in the adult population and are transmitted via the faecal–oral route. Infection may be through contact with an infected individual, through food or waterborne transmission or after contact with contaminated environmental surfaces. Noroviruses are member of the Caliciviridae and are non-enveloped small round structured viruses, 27–32nm in diameter. The genome consists of ssRNA and diversity within this group of viruses is associated with genetic drift and recombination. Currently, there are three norovirus genogroups and 15 genotypes.

The advent of new molecular and immunological methods for the diagnosis, characterisation and epidemiology of these virus infections allows a more systematic approach to their study. Sensitive and specific methods for detecting viruses in the environment and the detection of asymptomatic carriage may allow a clearer picture of the burden of disease associated transmission in the workplace.

Implications for hospital managers – Norwalk virus

Carl Dodd

Deputy Director for Clinical Services (Operations), Norfolk and Norwich University Hospital NHS Trust

In February 2003 the Norfolk and Norwich University Hospital had an outbreak of the Norwalk virus that lasted for 6 weeks, and had 211 active cases, affected 23 wards and had a major impact on the management of emergency and elective admissions.

The main specialties affected were Medicine for Elderly and orthopaedic surgery. The outbreak had a major impact on the Trusts ability to deliver NHS targets. In addition to patients, at its peak, the virus affected 102 members of staff, mainly nursing staff, which severely constrained the Trusts ability to cope with the outbreak.

There were many organisational and operational problems identified during this outbreak, including the isolation of affected patients, maintaining the day to day running of a large hospital and ensuring that the communication between infection control teams, operational managers and support services was clear.

From this, the Trust has learned many lessons on improving its resilience in responding to any subsequent outbreaks.

Smallpox

T. Brooks

Health Protection Agency, Porton Down

Abstract not received

Occupational health risks associated with working with rabies and other lyssaviruses

S.M. Brookes[1], G. Parsons[1], A. Rasey[2] & A.R. Fooks[1]

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Classical rabies virus (RABV) and other Lyssaviruses are zoonotic viruses capable of causing fatal encephalitis. Rabies can be prevented by pre-exposure vaccination involving a 3 dose course (D0, 7 & 28) and boosters at 3–5 years and/or rapid post-exposure treatment (PET) with immunoglobulin. However, once clinical symptoms develop PET is not effective.

Those at known risk of exposure to Lyssaviruses are laboratory staff and people in endemic countries. Following the detection of European Bat Lyssavirus (EBLV) type 2 in the UK in two Daubenton's

bats in 1996 and 2002 plus a human case in 2002, people who have contact with bats are also considered an at risk population.

Prior to this human EBLV-2 case, rabies vaccine was free through the NHS to licensed bat workers only. Data indicated that <50% *(n=193)* of all bat handlers had been vaccinated and 25% of those had lapsed. The vaccination rate has improved during 2003 as vaccine is now free to all bat handlers, however, vaccine responses are not currently monitored.

Vaccinated laboratory workers at the VLA were monitored and boosted following a blood test of <1.0 IU/ml anti-RABV neutralising antibodies (FAVN). An analysis of 25 laboratory staff indicated that 28% maintained a suitable antibody level for 5–10 years after last boost, but 12% had a titre of 1.0 IU/ml for <12 months. Generally, 60% required a boost vaccination in 2.5 years (±1.5). During 2002, internal VLA policy was changed to increase antibody monitoring from 2 to 4 times p.a. and to maintain a RABV titre of >5.0 IU/ml for those in contact with non-RABV Lyssaviruses.

Offered papers

Epstein–Barr virus can establish a persistent infection in the absence of a memory B cell population

M. Conacher[1], R. Callard[2], K. Mcaulay[1], H. Chapell[3], D. Webster[4], D.S. Kumararatne[5], G. Spickett[6] & D. Crawford[1]

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The primary site of Epstein–Barr virus (EBV) persistence is the B lymphocyte, but whether the virus resides exclusively in the memory B cell population is still disputed. We have analysed EBV infection in 9 cases of X-linked hyper IgM syndrome (HIM) who, due to a mutation in the gene coding for CD40 ligand, do not have memory B cell population.

We used a sensitive PCR technique to detect EBV DNA in blood and throat wash samples. Overall we found evidence of EBV infection in 66.7% of cases, which is similar to the level found in the general population (61% for a similar age range). We detected expression of EB small RNAs (EBERs) in peripheral blood in 2 cases, with additional expression of latent membrane protein (LMP)2 in one case and EB nuclear antigen (EBNA) 3C in another. In one case where 2 throat wash samples taken 6 months apart were available, we detected identical EBNA 3C sequences in both samples.

Our results show that a sustained EBV infection can occur in the absence of a memory B cell population. This finding could be explained by regular re-infection of the B cell pool either by lytic replication within the body or from the same external source.

Prevalence of human papillomavirus (HPV) in females attending a gum clinic in the West Midlands

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Background: HPV infections are among the most common sexually transmitted diseases especially amongst young adults and over 100 types have been distinguished.

Objectives: To determine prevalence of HPV in women attending a GU clinic; to investigate urine and chlamydia swabs as possible diagnostic samples; to examine co-infection with *C. trachomatis* in the <20's.

Methods used: A lightcycler based PCR with a confirmatory block PCR (Strauss *et al.* 1999). Paired urine/residual smear samples were collected from 210 women aged >20 and urine/residual chlamydia samples from 88 women <20.

Results: For <20's–18 urines and 21 swabs were invalid. 15/70 (21.4%) urines and 10/67 (14.9%) of swabs tested were positive. This represents 22 positive patients (31.4%). Three of the 70 patients were co-infected with HPV and chlamydia (4.3%).

>20's–86/210 urines and 28/210 smears were invalid. 7/124 (5.6%)urines and 22/182 (12.1%) smears tested were positive. This represents 25/193 (13.0%) patients with at least one sample positive.

Conclusion: Prevalence of HPV in the <20's (31.4%) was higher than in the >20's (13.0%). Urine samples were unacceptable as 30.8% were invalid. Co-infection with chlamydia in the <20's was less than expected (4.3%). Molecular epidemiology and results with reference to cytological findings are being analysed.

HPV detection in archival cervical cancer samples from Mauritius

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High Grade human papillomavirus (HPV), a sexually transmitted organism ia an important agent in the aetiology of cervical cancer, the most common form of malignant tumour in women worldwide. However, few studies have been carried out on specific racial groups. In Mauritius, cervical cancer accounts for 65% of gynaecological cancer and 3.4% of the cervical cancers were diagnosed at the stage of carcinoma *in situ*. In archival tissue collected from 65 patients diagnosed with cervical cancer, 19% were found to be positive for the presence of HPV 18 exclusively. HPV18 is believed to integrate into chromosomal band 8q24 that contains common fragile sites, regions of genomic instability that are associated with deletions, translocations, and gene amplification during cancer development. Only 15% of the Mauritian population is above 50 years of age, whereas 66% [35] of the diagnosed cases of cervical cancer were seen in patients above 50 years with 50% [5] affected with HPV. These findings are suggestive that for an infection with HPV to develop into cancer may take years if not decades.

Patients with post-herpetic neuralgia have elevated antibodies to neurofilament subunit

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Varicella zoster virus causes chicken pox, establishes latency and reactivates later in life to cause zoster (shingles). Up to 10% of patients with zoster develop post-herpetic neuralgia (PHN), which can result in debilitating severe chronic pain. Pathogenesis of PHN is poorly understood. This study investigated whether development of PHN is related to the level of antibodies to axonal cytoskeletal proteins.

We recruited 218 patients with acute zoster and followed them up for 1 year. IgG level to actin, tubulin and light, medium and heavy neurofilament (NF-L, NF-M and NF-H) subunits was measured by

enzyme immunoassay in serum samples obtained at the onset of zoster rash. The results of 26 cases with PHN at 3 months and 26 age and immune status matched controls were analysed using Fisher's exact test.

No significant variation was seen in antibody levels to actin, tubulin and medium and heavy neurofilament subunits between the two groups. However, antibody level to the 68 kDa light neurofilament (NF-L) subunit was significantly elevated in patients with neuralgia (p=0.024). Anti-NF-L antibodies seem to be raised in patients with acute zoster, who later developed PHN and may contribute to nerve damage with persistence of pain and/ or altered sensation. They may serve as a marker to identify patients at increased risk of PHN and aid targeted antiviral therapy.

External quality assessment for the molecular detection of hepatitis C virus

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Since the discovery of hepatitis C virus (HCV) in 1989 the advent of blood donor screening has reduced the incidence of acute infection. However, asymptomatic carriage and infection are common and chronic infection can lead to increased risk of cirrhosis or hepatocellular carcinoma. Millions of people are known to be infected with HCV, with intravenous drug users and transplant patients at particular high risk. External quality assessment schemes provide valuable information regarding methods, standardisation and the success in detecting and reporting infections in clinical diagnostic laboratories. The UK National External Quality Assessment Service for Microbiology provides an EQA scheme for molecular detection of HCV RNA in which participants are asked to determine the presence, genotype and quantity of HCV RNA in freeze dried plasma specimens. An up to date summary of the data from the EQA scheme for HCV will be provided. Standards of HCV molecular diagnostics remain high among participants in the scheme, with 86.9% to 100.0% laboratories detecting HCV depending upon viral load and 92.6% to 100.0% correctly identifying the genotype. Improvements have been observed in the inter-assay variability and in the standardisation of reported units with the number of laboratories reporting in IU/mL increasing from 46.9% to 97.3%.

Evaluation of liquid based cytology (LBC) for the detection of HPV by PCR

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Around half million new cases of cervical cancer diagnosed worldwide each year accounting for almost 300,000 deaths. Development of cervical cancer can be multi-factorial, but high-risk human papillomavirus (HPV) have been associated with the aetiology of cervical cancer. It is believed that HPV integrates into the host DNA causing abnormal cell growth with cells becoming carcinogenic and spreading metastatically. In the UK alone there are, on average, 3200 new cases yearly resulting in about 1200 deaths.

Pap smear remains the most common method for the detection of pre-cancerous changes in cervical cytology. The introduction of a liquid based cytology (LBC) method expands the possibility of diagnosis of cervical intraepithelial neoplasia (CIN). LBC allows detection of pre-cancerous changes and human papillomavirus (HPV) simultaneously. Detection of HPV by PCR from LBC has not been reported in the literature. We therefore undertook an evaluation study to establish whether LBC was appropriate for HPV detetction by PCR.

In our evaluation study using an in-house PCR method, high grade HPV was detected in 32% of the 38 LBC tested. The results tallied with cytology findings. The results obtained suggest that this conventional PCR method could be developed for use on a real-time PCR platform or in a microtitre well format and subsequently automated for the detection of HPV in LBC.

An outbreak of hepatitis B in an IDU and non-IDU population

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Background: Reports of acute hepatitis B in Bristol, UK have been increasing since August 2001 (Table 1). In 2002 the rate/incidence of lab reported acute hepatitis B was 7.6/100 000, over three times the rate for England.

Table 1. Laboratory confirmed HbcIgM positive cases in Bristol, 1999–June 2003

	1999	2000	2001	2002	2003*
Number of cases	19	15	27	75	34

**Cases to June 2003*

Cases increased first in male injecting drug users (IDUs) from mid-2001. IDUs make up about forty percent of cases in the outbreak.

Methods: Molecular Epidemiology

Amplification was carried out over the HBsAg and HBcAg coding region. All PCR positive samples were sequenced using the PCR internal primers, then analysed using the DNASTAR software. Phylogenetic analysis was undertaken.

Case Control Study

Cases were defined as having acute hepatitis B clinically (HbsAg+ve and HBc IgM+ve) and a history of IDU. IDU controls were recruited through community outreach. Both groups were interviewed. Non-IDU cases have also been interviewed to identify risk factors.

Results: Molecular Epidemiology

We have identified a sequence not seen in an outbreak in the UK in 43/50 specimens so far.

Case Control

We have interviewed 124 community IDU controls and are contacting 40 IDU cases.

Conclusions: We describe an outbreak of hepatitis B amongst IDUs with apparent heterosexual spread into the non-injecting population.

Monitoring the progress of BK virus associated nephropathy after renal transplantation

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Nephropathy associated with BK virus (BKVAN) has recently emerged as an important cause of allograft failure following renal transplantation. The aim of this study was to determine the

relationship between urine and serum BK viral load during follow-up of patients with this condition and compare them to urine electron microscopy (EM) and cytology. Serial samples from seven renal transplant patients with biopsy proven BKVAN were studied. The median follow-up time from diagnosis was 76 weeks. Interventions included immunosuppressive dose reduction, drug changes and/or the use of the antiviral agent cidofovir. Serial urine samples (n=127) were collected for EM, decoy cell detection and quantitative urine BK viral load using real-time polymerase chain reaction. Serum BK viral load was also measured serially (n=72). All patients showed a reduction in serum and urine viral load over the period of follow-up co-incident with the loss of decoy cells and negative EM result. Overall, urine samples that were negative for decoy cells or EM had the corresponding urine viral load less than 106 copies/ml and serum viral load less than 103 copies/ml. In paired serum/urine samples, there was a proportional relationship between serum and urine viral load with each urine viral load approximately three orders of magnitude greater than its corresponding serum level. In transplant centres where routine quantitation of BK virus DNA is not available, negative EM and absence of decoy cells could be used as broad indicators of a response to intervention. However, measurement of BK virus DNA level provided a wider dynamic range and should be the method of choice for determining the extent of viral control. The relative merit of urine or serum viral load need further studies.

Education & Training Group session

Bored with same old culture? Inoculate new life into your classroom!

Introduction... but I've been doing it this way for 20 years!

S. Assinder

University of Wales, Bangor

Recent years have seen an increase in the number and range of resources available for teaching microbiology, yet teachers are sometimes reluctant to move away from tried and trusted methods. This talk will give an overview of some of the resources available, including those produced by the SGM, and will show how these can be used to enhance the teaching and learning of microbiology.

What microbiology do they learn at school?

John Grainger

Microbiology in Schools Advisory Committee, Marlborough House, Basingstoke Road, Spencers Wood, Reading RG7 1AG

It is regrettable that as the significance of microbiology to everyday life increases, there is now less microbiology in school syllabuses than when the National Curriculum was launched. Nevertheless, microbiological topics still feature in most key stages and at 16+ and teachers are keen to teach them. However, teachers often have a limited background in microbiology and feel the prospect of practical work particularly daunting. Textbooks and the internet are ready sources of theoretical material for general needs but guidance is needed for access to specialised aspects, particularly at 16+. Obstacles to practical work are concern at safety issues, lack of technical skills among teachers and technicians, and the need for protocols appropriate for particular levels.

Fortunately there are several sources of help available to schools and growth in their significance is encouraging. Some are general or specialised science centres; others work within a framework provided by professional organisations, learned societies, educational institutions and charitable bodies, often with key voluntary input. SGM has developed a leading and increasing role in supporting microbiology in schools, both directly and as a major sponsor of the Microbiology in Schools Advisory Committee since its foundation in 1969. (See www.microbiologyonline.org.uk/misac).

Use of the Personal Response System (PRS) in bioscience teaching

M. Mattey

University of Strathclyde

Teaching problems with large classes are well known, the traditional 'top down' lecturing approach frequently does not deliver what either the lecturer or the student want. Interactive teaching, where a more Socratic question and answer session occurs, is an alternative, but one that seemed unattainable in large classes with limited staff resources and time.

PRS is a tool for effecting electronically immediate feedback and reinforcement for active learning by all students in the classroom.

Surveys of student users indicate that a majority of them consider the following features as valuable:

- answering in private;
- discussion with peers before answering; and
- knowing how their peers responded.

Each participant responds privately to a question or prompt, all responses are collected, and the summary displayed for all to see.

With questions in a multiple-choice format, participants answer by pressing a number on their pocket-size wireless transmitter. The signals sent are processed by a portable receiver that in turn is connected to a central display via a PC. Each signal from a transmitter causes one of the boxes shown on the display to change color. The answer itself is not displayed. At the end of the question period, a statistical summary of the answers is shown on the display as a histogram.

This session will use interactive methods and examine student performance outcomes.

Something for everyone? A whistlestop tour of ideas and resources for lecturers

H.J. Sears

Learning & Teaching Support Network Centre for Bioscience, Leeds

In the pressured world of higher education it can be very difficult to find the time to change any aspect of what we do – no matter how much we may want or need to. The focus of the short session will be to showcase a range of ideas and resources that have been developed to help bioscience lecturers adapt their teaching practice and support student learning. LTSN Bioscience can be contacted on Tel. 0113 343 3001, Email ltsnbioscience@leeds.ac.uk and on the web at http://bio.ltsn.ac.uk/.

Novel assignments: when students become module leaders

Olivier A.E. Sparagano

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Following the lack of interest of our students for microbiology/parasitology modules and the redundancy in assessment methods within the same degree course I decided to change my modules implementing peer and self-assessment methods through the preparation of a parasitology conference. The final year students selecting the parasitology module have six months to organise a parasitology conference for which they have, almost, the entire responsibility. Students decide whom they want to invite as guest speakers, find their own sponsors and advertise the conference as they wish. Two marks are given to them for (1) their scientific presentation (usually within a 4–5 student group) alongside their guest speakers and for the conference organisation (1 mark returned for the whole class). Both marks are coming from the public and are then altered by the students themselves after peer- and self-assessment sessions. The self-assessment exercise showed that top students are very often downgrading themselves and weak ones are denying their lack of participation/performance; whereas the peer-assessment exercise (within a student group presenting a talk) shows different marking behaviour: either the students stick to each other without (if necessary) downgrading or upgrading some of them or by they downgrading each other to save their own mark. In exceptional cases there is no consensus between the students 'shooting' at each other.

Innovative teaching method: peer assessment

S. Maw

LTSN Bioscience, University of Leeds

The assessment of student work by other students has many potential benefits to learning, both for the assessor and the assessee. Such practice encourages student autonomy and higher order thinking skills. This presentation will outline examples (laboratory reports, posters, short answer questions and essays) of peer assessment from pharmacology which has been used up to 200 students. The talk will be a realistic approach to peer assessment highlighting the benefits, restrictions and some pitfalls to avoid. In addition, data to support the claims of improved learning will be presented.

Novel assignments: communicating microbiology to the outside world

J. Verran

Dept of Biological Sciences, Manchester Metropolitan University

The development of transferable skills is now either implicit or explicit in most undergraduate curricula – for example, students may complete portfolios whereby the 'skills element' of a given assignment is separated from the 'science' to enable demonstration of a given 'skill'. Oral presentations, group work, time management, writing skills etc. are embedded into the activities which students carry out.

An interesting slant on these activities involves the communication of scientific ideas or information to a different audience. At MMU, a number of such projects have been completed: students have been involved in the production of public information leaflets or posters; videos for use in schools; learning activities to accompany museum exhibits; interdisciplinary activities with artists; production and evaluation of games, and so on.

In all cases, projects have been completed, and generally enjoyed by the students. The product provides evidence of successful completion of the project, and the novelty provides an ideal opportunity for the student to explore and describe the acquisition of transferable skills. The strength of the activities lie in the external links: the projects are real, the clients have a need, and both the client and the intended audience are involved in the evaluation of the product. Projects lacking these links utilize aspects of microbiology whose immediacy and general availability provides relevance, for example by the collation of news stories and evaluation of the language used by the press, or by reading selected novels and reviewing microbiological aspects within.

The activities provide an unusual adjunct to the degree programme, and their diversity provides flexibility in implementation. They have been used as module based, assessed course work, alternative to placements, final year project work, and other student centred learning activities. The presentation will outline some of these activities in more detail.

American Society for Microbiology resources for higher education

Erica Suchman

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My talk will introduce participants to the many resources for Microbiology Educators provided by The American Society for Microbiology (ASM). Division W is a branch of the ASM that hosts sessions at the yearly ASM general meeting covering various topics pertinent to microbiology Educators, as does the Board of Education and Training (BET). The BET also runs a yearly Microbiology Educator's Conference dedicated solely to microbiology education. All of these meetings are open to both ASM members and non-members, and the Microbiology Educator's conference is always run the 3 days preceding the general meeting so that participants can easily and inexpensively attend both meetings. Furthermore the BET also coordinates the MicrobeLibrary (www.microbelibrary.com) a searchable portal providing a peer-reviewed, web-based collection of over 700 resources about the microbial world. The goal of the MicrobeLibrary is to provide microbiology educators with a collection of educational resources, including visual images, laboratory and classroom activities, assessment tools, and microbiology education articles, that are correlated to key microbial concepts that the ASM has identified as necessary for building a strong microbiological program. All published resources are reviewed for both scientific accuracy and instructional value. In 2002, the MicrobeLibrary received an average of 22,650 visitors per month.

Environmental Microbiology Group / British Mycological Society joint session

The role and impact of fungi on biogeochemical cycles

Geomicrobiology: relative roles of bacteria and fungi as geomicrobial agents

Henry L. Ehrlich

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Geomicrobiology is a study of the role that microbes have played in the past and are currently playing in some processes of fundamental importance in geology. The microbes of interest include various members of the prokaryotic domains Archaea and Eubacteria, and the fungi, algae and protozoa in the eukaryotic domain. Of the eukaryotes, only the fungi will be considered in this discussion. Geological processes that are microbially influenced include some forms of mineral formation, mineral degradation (e.g., weathering, soil and sediment formation and transformation, metal mobilization or bioleaching), elemental cycling, chemical and isotopic fractionation, and fossil fuel genesis, exploitation, and degradation. In many cases, an association of micro-organism that may consist only of prokaryotes or eukaryotes, or of a combination of prokaryotes and eukaryotes may be involved. Many prokaryotes affect geochemical processes enzymatically, whereas the large majority of fungi affect geochemical processes non-enzymatically. The reason for this is differences in cell organization. Geologically active microbes may exert their effect through (1) enzymatically catalyzed processes, mainly inorganic and organic oxidations or reductions, (2) action of some of their excreted metabolic products including organic and inorganic acids, alkaline substances, and/or ligands, (3) cell surface interactions, (4) physical effects exerted by biomass, or (5) a combination of these. Some of these geomicrobial activities will be illustrated in other presentations in this symposium.

Fungal dissolution and transformation of minerals: significance for nutrient and metal mobility and contrast with bacterial mechanisms

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In terrestrial environments, fungi serve as important geochemical agents although are more usually associated with carbon cycling due to their role in organic matter decomposition. However, fungi function as agents of biogeochemical change in a much wider context being involved in cycling of many other elements. Fungi promote rock weathering and contribute to the dissolution of mineral aggregates through excretion of H^+, organic acids and other ligands, or through redox transformations of mineral constituents. We have found that the main mechanism of metal mobilization from insoluble metal minerals is a combination of acidification and ligand-promoted dissolution mediated by different organic acid anions. However, if oxalic acid is produced the production of metal oxalate minerals may result. We have also shown that fungi may play an important role in the transformation of micro-fabrics in limestone ($CaCO_3$) and dolomite ($CaMg(CO_3)_2$) and have produced direct evidence of mineralized fungal filaments with secondary carbonates. Other experiments using laboratory microcosms showed that fungi can precipitate calcite ($CaCO_3$) and whewellite (calcium oxalate monohydrate, $CaC_2O_4.H_2O$). Fungi therefore play an active or passive role in mineral formation through precipitation of secondary minerals, e.g. oxalates, and through the nucleation of crystalline material onto cell walls that can result in the formation of biogenic micro-fabrics within mineral substrates. Such interactions between fungi and minerals are of importance to biogeochemical cycles for metals as well as those of C, H, O, N, P and S.

Importance of nutrient deficiency on dissolution of minerals by ectomycorrhizal fungi in the field

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Different minerals have been shown to change in structure and chemical composition after exposure to ectomycorrhizal fungi. These interactions may take place, as the fungi require nutrients from the minerals but it is also possible that the influences on the minerals are results of other actions by the fungi not related to nutrient acquisition. These questions are important to address for future studies since the answers will have implications for long-term sustainability of forest ecosystems.

We have found that a forest exposed to P deficiency allocated more resources to a P source (apatite) in the soil. Ectomycorrhizal roots from the P deficient forest accumulated more rare earth elements originated from the apatite suggesting that transport rates from the apatite to the trees was higher in the P deficient forest. The results suggest that P deficiency induced by increased biomass harvesting to some extent can be compensated for, by increased weathering induced by ectomycorrhizal fungi. No similar exploitation of K sources (biotite) was found in forests exposed to K deficiency and we could not detect increased transport of elements from biotite to the trees in these forests. This suggests that the forests are less adapted to K deficiencies, which may develop in sites with high harvesting rates.

Phosphate solubilization

J. Berthelin

CNRS, Vandoeuvre les Nancy, France

Abstract not received

Fungal activities in rock-inhabiting microbial communities

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Rock surface colonisation is an important starting point in the development of all terrestrial ecosystems on Earth. The interface between the rock substrate and the atmosphere is inhabited by a complex microbial community including chemoorganotrophic – fungi and bacteria – and phototrophic – green algae and cyanobacteria – micro-organisms. Sub-aerial rock communities metabolise under a limited water availability and high sun irradiation levels and can be found even on desert rocks and high mountain altitudes. Sub-aerial epi- and endolithic biofilms create and maintain biologically modified environments where mineral solubility and dissolution rates are significantly altered. Fungi are the most persistent inhabitants of the subaerial rock biofilms due to their hyphal and/or yeast-like

growth mode and remarkable stress tolerance of certain ecological groups. However, in complex subaerial biofilms free living fungi frequently maintain mutually beneficial contacts with bacteria and phototrophic organisms. Fungal influence on solid rocks is manifested through (i) a mechanical impact on grain cohesion through turgor pressure, (ii) a series of excreted secondary metabolic products (low molecular weight organic acids and organic polymers including diverse pigments and antioxidants) and (iii) biologically induced formation of new minerals (carbonates, oxalates, oxides, phosphates and silicates or silica). Further geomicrobiological and biogeochemical role and modifications of fungal metabolic products on rock surfaces will be discussed.

Roles of bacteria and fungi in carbonate–oxalate biomineralization

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The role of Fungi and Bacteria in the calcium-carbon cycle in soils and surficial sediments is much more important than is commomly believed. The African iroko tree (*Milicia excelsa*, Moraceae) constitutes a remarkable natural laboratory to study the relationship of oxalate biosynthesis and calcium carbonate precipitation under the influence of Plants, Fungi, and oxalotrophic Bacteria in a calcium carbonate-free environment. Oxalic acid as well as oxalate crystals (as whewellite) are produced by the tree during its life. When wood is decayed, large amounts of oxalic acid are produced by Fungi and accumulate as calcium oxalate (as weddellite). In addition, wood rot Fungi release whewellite crystals from their organic matrix during wood decaying. Both sources of oxalate are oxydized by oxalotrophic bacteria inside the tree (by wood endophytes) and in the surrounding soil. The transformation of oxalate into carbonate increases the soil pH. In an oxalate-free ferralitic soil, the pH is usually 4.9–6. Under the iroko, the pH reaches 8.9, making the environment stable for calcite precipitation and preservation. Moreover, some fungal strains directly precipitate needle fiber calcite deeper in the soil, leading to even more calcium carbonate storage. Both laboratory cultures and biogeochemical theoretical studies demonstrate that the oxalate-carbonate cycle may constitute an important carbon sink.

The role of arbuscular mycorrhizal fungi in carbon and nutrient cycling

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Arbuscular mycorrhizal (AM) fungi are major components of soil microbial biomass and have key functional roles in carbon and nutrient cycling and in influencing the composition of plant communities. Despite this, we know little about the functional roles of the extra-radical mycelium of AM fungi, which, due to its cryptic habit, is often the 'hidden half' of the symbiosis. Recent estimates suggest that the external mycelium can extend to 100 m per gramme of soil in some grassland and can receive up to 10% or more of the net photosynthate of their host plants. This review highlights the evidence of the amount of carbon allocated to AM mycelial systems in temperate grassland, and its subsequent use for biomass production, respiration and as the energy source for uptake of plant limiting nutrients, and the techniques that are now available to understand more fully the functioning of AM fungi in the field. We also highlight the multi-functional nature of the external mycelium in terms of its interactions with other soil organisms and its role in influencing plant community composition.

Fungal roles in transport processes in soils

Karl Ritz

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Fundamentally, biogeochemical cycling involves the *transformation* of compounds between various forms, and a *movement* of such compounds within and between compartments of the biosphere and geosphere. These processes operate across a vast range of spatial and temporal scales. In terrestrial systems, transformations and movement of materials belowground are governed by the spatial organisation of the soil system, and particularly the architecture of the pore network. This 'inner space' provides the physical framework in and through which the majority of soil-based processes occur. The labyrinthine nature of the pore network, and the exchange properties of associated surfaces, strongly regulates the transport of material through the soil matrix.

Soil organisms play a key role in driving nutrient cycling, and fungi contribute a wide range of functions relating to such cycling processes. Eucarpic fungi are well adapted to operating in spatially structured environments, since the filamentous growth form allows an effective exploration of space for resources, and an exploitation of such substrate when located. Furthermore, materials can be transported within mycelial networks and hence through the soil matrix, potentially short-circuiting prevailing physical pathways. This presentation will review how the spatial organisation of physical and nutritional factors affect mycelial morphology of soil fungi, the transport of materials via such networks, and the implications for nutrient cycling in soil systems.

Rock-eating mycorrhizas

Mark M. Smits

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In 1997 tunnels were discovered in feldspar grains. These tunnels are thought to be a result of organic anion excretion by soil fungi, specifically ectomycorrhizal (EcM) fungi ('rock-eating mycorrhizas').

In contrast with other biotic weathering phenomena, tunneling can be quantified by image analysis.The contribution of this fungal tunneling to weathering of the uppermost mineral soil across a North Michigan dune chronosequence was studied. Results show that the contribution of fungal tunneling to feldspar weathering is low. Fungal tunneling seems to play a minor role in both feldspar weathering and ecosystem influx of Ca and K. The total impact of fungal activity on feldspar weathering remains to be ascertained, because tunneling is only one aspect of fungal weathering.

'Rock eating mycorrhizas' probably also transport weathering products like aluminium to the plant root. Indeed budget studies suggest a biotic flux of aluminium from the mineral soil layer into the organic soil layer of podzols, where most of the roots are. Prelimary results show that *Paxillus involutus*, an EcM fungus, transport aluminium in a two-compartment agar system.

Integrated nutrient cycles in forest ecosystems – the role of ectomycorrhizal fungi

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Ectomycorrhizal fungi have direct access to a supply of energy-rich host assimilates which enables extensive mycelial proliferation within both the organic and mineral horizons of forest soils. Studies of ectomycorrhizal distribution are almost always confined to the upper organic horizon. However, we recently found that over half of the

ectomycorrhizal species in one forest soil occurred exclusively in the underlying mineral horizons. Laboratory studies of ectomycorrhizal mycelia colonizing different mineral substrates *in vivo*, or in plant microcosms, also suggest that patterns of mycelial growth, carbon allocation and substrate acidification may be influenced by mineral composition. It is increasingly evident that different groups of ectomycorrhizal fungi have the capacity to mobilize N and P from microbial, micro- & meso-faunal biomass, as well as plant litter. However, growth of fungal mycelia and allocation of carbon compounds to their hyphal tips also has important consequences for acquisition of base cations, tolerance of Al and heavy metals and weathering of different mineral substrates. Communities of ectomycorrhizal fungi thus play multiple roles in mobilising and transporting both mineral and organic nutrients to their host plants. Further research is still needed to investigate the role of carbon supply through ectomycorrhizal mycelia and how this influences other microbes, especially with respect to the complementary roles these may play in mobilisation of inorganic and organic nutrients.

Toxic metal mineral transformations in the mycorrhizosphere

Marina Fomina[1], Stephen Hillier[2], Ian J. Alexander[3] & Geoffrey M. Gadd[1]

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The physico-chemical environment of the mycorrhizosphere is different from that of the bulk soil and this can affect toxic metal speciation. The mechanisms by which fungi and plants obtain phosphate in the mycorrhizoshere are of special interest since solubilization of inorganic phosphates can result in release of associated metals. The aim of this research was to study toxic metal mineral transformations by ericoid and ectomycorrhizal fungi in axenic culture and in mycorrhizal association. Of the tested insoluble cadmium-, copper-, lead- and zinc-containing minerals, zinc phosphate was of least toxicity and the most easily solubilized by mycorrhizal fungi. The main mechanism of metal mobilization from insoluble metal minerals was found to be a combination of acidification and ligand-promoted dissolution mediated by different organic acid anions: if oxalic acid was produced, the formation of metal oxalates resulted. A study of zinc phosphate solubilization by ectomycorrhizas in a mesocosm experiment showed that in the presence of a phosphorus source, an ectomycorrhizal association using a zinc-tolerant *Paxillus involutus* accumulated much less zinc than the non-mycorrhizal plants (*Pinus sylvestris*). In contrast, in the absence of a phosphorus source a zinc-tolerant mycorrhiza considerably increased zinc mobilization and accumulation and maintained a high phosphorus concentration in mycorrhizal roots.

Relative roles of bacteria and fungi in polycyclic aromatic hydrocarbon biodegradation and remediation of contaminated soils

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Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous contaminants whose environmental fate is of increasing concern because of their known or suspected mutagenic, carcinogenic and ecotoxic potential. PAH degradation by microbial systems is a very promising technology to remediate PAH contaminated terrestrial and aquatic ecosystems. A variety of bacterial and fungal species in axenic culture have the ability to metabolize PAHs with three rings (phenanthrene, anthracene), four rings (pyrene, benz[*a*]anthracene) and to a limited extent five rings (benzo[*a*]pyrene) to CO_2 or metabolites that are considered dead-end products. It is generally viewed that the recalcitrance of high molecular weight PAHs is primarily due to very low solubility and the hydrophobic nature of PAHs resulting in their partitioning into the soil matrix, thereby limiting their bioavailability for microbial degradation.

There are similarities and differences in the mechanisms of PAH metabolism used by procaryotes and encaryotes. The microbial degradation pathways have been described and the enzymatic mechanisms elucidated. Bacterial degradation of PAHs under aerobic conditions begins with oxidation of the aromatic ring catalyzed by dioxygenases to *cis*-dihydrodiols. These compounds are subsequently dehydrogenated to form dihydroxy-PAHs which may be substrates for ring-fission enzymes. Ligninolytic and non-ligninolytic fungi and yeasts have been shown to degrade a wide array of PAHs. Many fungi (*Cunninghamella, Penicillium, Aspergillis* species) oxidize PAHs catalyzed by cytochrome P450 and epoxide hydrolase to form trans-PAH dihydrodiols. These reactions are highly regio- and stereoselective and are generally similar to those used by mammalian enzyme systems. The white-rot fungi and wood decaying basidomycetes (*Phanerochaete*, *Pleurotus*, and *Trametes* species) catalyzed the initial attack on PAHs by producing extracellular enzymes such as lignin peroxidases, manganese peroxidases or laccases to form PAH diphenols which are oxidized to quinones. These intermediates can undergo ring-fission to produce CO_2.

Although there is considerable information on the degradation of PAHs under laboratory conditions with pure cultures, field experiments in PAH contaminated soils have shown that high molecular weight PAHs such as benz[*a*]anthracene, benzo[*a*]pyrene and chrysene resist extensive degradation in soils and sediments. However, recent studies have indicated that bacterial and fungal co-cultures significantly increased the rate of degradation.

An overview of current research and recent findings on the microbial degradation of PAHs will be presented. Our current understanding of the demonstrated applicability of bioremediation to decontaminate PAH contaminated soils and sediments to appropriate clean-up levels will be discussed.

Fermentation & Bioprocessing Group session

Meeting the challenges of producing proteins and antibodies

Applications of SELDI ProteinChip® technology for protein expression analysis and downstream purification optimization

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SELDI (Surface Enhanced Laser Desorption Ionization – Mass Spectrometry is a novel technology that combines miniaturized chromatographic capture and time-of-flight mass spectrometric detection, which can be applied to the analysis of crude feedstreams.

The technology relies on protein biochip arrays carrying functional groups (e.g. ion exchange, hydrophobic interaction, reverse phase, IMAC). These arrays have been designed to bind proteins and peptides from fermentation or cell culture supernatants.

SELDI allows selective sample binding/desorption on functionalized surfaces followed by mass spectral analysis of retained components.. This molecular weight-based analysis also reveals information about protein integrity and behaviour that can be exploited for the purpose of process optimization.

Applications of SELDI as a fast selectivity screening tool for downstream chromatographic purification and expression analysis will be discussed.

Affinity, metal chelate and ion exchange chromatography on Sartobind Membrane Adsorbers

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Membrane adsorbers have gained increasing acceptance in the field of bioprocessing during the last years [1]. Besides numerous systems for laboratory scale applications, a modular system of membrane adsorbers for a technical scale has been reported [2].

This technology with its growing range of affinity ligands is of increasing importance for pharmaceutical down stream processing, encompassing the open structure of membranes with the benefits of classical chromatography.

A short overview of the units, the mode of operation and applications in different fields of biotechnology and bioprocessing are presented. The main applications to date are ion exchange processes for polishing and capture of different biomolecules.

New types membranes can be incorporated into such units. Examples are Protein A, para-aminobenzamidine and iminodiacetic acid as ligands attached to the membrane. Applications of these membranes on a small scale are shown.

Furthermore a new series of disposable units with different types of membrane adsorbers is presented. Selected features of this new units regarding number of layers, mode of operation, and physico-chemical stability are presented.

As a future application chemically activated membranes for enzyme reactors are presented. The advantages of the convective flow through membranes for such applications are discussed.

References: [1] Gosh, R. (2002). *J Chromatogr A* **952**, 13–27 / [2] W. Demmer & D. Nußbaumer (1999). *J Chromatogr A* **852**, 73–81.

Integral membrane proteins: theory, problems, and some solutions

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The biological membranes of bacteria are jam-packed full of proteins. Indeed, recent genome analyses confirm that up to one third of all cellular proteins may be associated with the membranes. However, because of both perceived and real difficulties with handling integral membrane proteins, our knowledge of their structure and operation has lagged-behind that of their water-soluble counterparts. In recent years, partly thanks to some timely initiatives to fund membrane protein research, our ability to harness these slippery customers has improved considerably and as a result our understanding of membrane protein biochemistry is also on the up. First, biosynthesis of biological membranes requires the controlled targeting and integration of proteins into lipid bilayers. We will consider the key features of this process. Next, in order to study the structure and function of an integral membrane protein it must be freed from the lipid bilayer and purified to homogeneity. We will consider the various detergents and other media required for these processes. Finally, we will review the state-of-the-art technologies available for the characterisation of integral membrane proteins.

Inclusion bodies – purification aid or obstruction?

J.M. Liddell

Head of Process Science, Avecia Biotechnology

Expression of heterologous proteins in *E coli* can frequently result in accumulation of the heterologous protein in an insoluble form – commonly termed an inclusion body.

Recovery of a soluble, active protein from the inclusion body requires additional process steps to recover the protein. Although the underlying processes involved in protein denaturation and refolding have been long understood, detailed understanding is lacking. Thus in spite of extensive study over many years refolding of proteins from inclusion bodies is an unpredictable process that must be considered on a case by case basis. These processes are multistep, generally need to be carried out at low concentration, poorly understood in detail and highly system specific The need to operate inclusion body processes at low concentrations mean that tanks necessary to carry out refolding processes are large and may require fabrication of special materials

To recover active protein from an inclusion body will generally require the following steps to be carried out:

- Isolation of the inclusion bodies from the cell – cell disruption and centrifugation
- Solubilisation of the inclusion body protein using a chaotrope (denaturant) under reducing conditions (to separate all disulphide bonds);
- Recovery of active protein by removal of denaturant under conditions that allow the protein to adopt its native configuration;
- Disulphide bonds, if present in the structure, need to be re-oxidised under controlled conditions achieving the correct configuration present in the native structure.

A number of factors are known which can impact on inclusion body refolding efficiency including redox state, protein concentration, overall purity, presence of specific impurities and additives such as amino acids, salts, sugars and solvents. As a result yields of correctly folded protein can be significantly different depending on the protein with yields ranging from over 60% to below 10% being reported.

Other, less recognised difficulties with the production of proteins in inclusion bodies include:

- Poor fit to screening protocols for active biopharmaceuticals;
- Difficulties in determining product expression levels in fermentations;
- Additional steps needed to distinguish inactive (misfolded) from active authentic product (correctly folded);
- Large process size due to low achievable product concentrations.

These disadvantages for inclusion body systems need to be set against a number of advantages that include:

- Inclusion bodies are highly pure (ca 60%) so that physical separation achieves a high purity material as feedstock to the purification process;
- Inclusion bodies are highly stable and compact. They can be stored (–20°C or lower) for many months providing a way of decoupling fermentation from purification;
- High productivity of *E coli* fermentation systems using simple media – titre per unit time;
- Provides a highly stable form of the protein providing physical segregation from cellular proteases.

Features of inclusion body isolation, solubilisation and refolding in relation to the production of biopharmaceutical proteins will be discussed. Considerations in developing refolding strategies for inclusion bodies will be described as well as recent work to aid development and optimisation of refolding processes.

References: Folding and association of proteins, R. Jaenicke, *Proc Biophys Mol Biol* **49**, 1987, 117–237 / Advances in refolding proteins produced in *E. coli*, H. Lilie, E. Schwarz, R. Rudolph, *Curr Opin Biotechnol* **9**, 1998, 497–501 / Preparative protein refolding, A. Middelberg, *Trends Biotechnol* **20**, 2002 437–43.

Clarification, concentration, and capture: a one-step event

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Traditionally, purification schemes of proteins produced by bacteria, yeast, mammalian cells, insect cells, plant cells and other biological sources, include centrifugation, filtration and one or more chromatographic steps. This approach can result in longer process times and reduced recovery. Expanded Bed Adsorption (EBA) eliminates the need for particulate removal prior to adsorption of the target protein. In EBA, a stable fluidized adsorbent bed is created by applying an upward liquid flow to the column. The expansion of the adsorbent creates a distance between the adsorbent particles, which allows for unhindered passage of the particles during application of crude feed to the column, while the target protein is adsorbed. After washing, the captured proteins are eluted. The eluate contains a concentrated, clarified, partly purified target protein, ready for further purification by packed bed chromatography. Major benefits of EBA are: decreased number of process steps, increased yield, shorter overall process time, reduced labor and reduced running cost, providing increased process economy. The latest developments will be presented and include: screening of the biomass-bead interaction, a new high density, salt tolerant gel and new columns.

The influence of scale of operation on purification process design

Jim Davies

Biochemical Engineer, Purification Development, LONZA Biologics plc

Monoclonal antibodies are expressed at comparatively low levels, typically less than 4 g l^{-1} and doses are disproportionately high. Therefore for late phase clinical trials and in market supply, such products need to be manufactured at large scale (100's of kg). In order to meet the increasing demand, Lonza has recently expanded its capacity to three 20,000 L fermenters with the associated purification trains, capable of producing over 2 tonnes of MAbs per annum.

Facility design and operation is dependant on the scale. Pilot scale requirements of a few hundred grams of MAb can often be satisfied with flexible, disposable facilities which reduce the high capital costs linked to fixed vessels and equipment. In this way the complexity of operation can be reduced which also minimises the numbers of utilities required. Understanding the design of the plant eases the transfer of processes between scales and can overcome some of the pit-falls associated with scale-up.

Examples of processes developed in the laboratory are discussed along with the implications on operation at large scale. Materials handling and waste disposal issues are magnified and the repercussions of this are recognised. Optimising processes to minimise costs are also considered with respect to raw materials and manufacturing schedule constraints.

A robust process – from bench to product

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One of the most important properties of a production process is that it should be robust i.e. capable of performing each time it is run with few or no failures. In order to save time and costs, it is important that robustness is built into the process from early in its development. However, it can be the case that a lack of understanding of the constraints of process-scale operation results in the development of an unsuitable process. An example will be shown of how this occurred and of how it was resolved. The use of experimental design and statistical modelling tools for the design of a process and how this can aid the creation of a robust process will also be shown.

Recent updates on protein A media

Anders Ljunglöf, Annika Andersson, Tryggve Bergander, Hans J. Johansson, Karol M. Lacki & Gunnar Malmquist

Protein Separations R&D, Amersham Biosciences, Uppsala, Sweden

Annual production requirements of antibodies for therapeutic use are in the order of metric tons. As a consequence, enhanced production capacities are required to handle 50–100 kg batches. Most production methods for monoclonal antibodies involve initial capture by Protein A affinity chromatography. Development of future affinity media for antibody production is to a large extent driven by process economics considerations. One line of research is directed toward development of a new Protein A media with significantly increased dynamic binding capacity. Another line of research involves genetic engineering of the affinity ligand to increase the alkaline stability. The ability to use 0.5 M sodium hydroxide would greatly facilitate the design of robust and inexpensive cleaning and sanitization protocols. Recent results obtained with prototype materials from the two separate research areas will be presented.

Engineering antibodies for biosensor technologies

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Biosensors can be used to provide real-time detection of biological warfare agents, gaining their specificity and sensitivity from antibodies incorporated into the biosensor. Polyclonal antibodies are easy to produce, but often carry the disadvantage of low specificity for their targets, with the attendant problem of false positives. Monoclonal antibodies can provide an improved specificity, although with a high cost of production. In an ideal world, reagents for biosensors would be cheap, robust, specific and highly sensitive. We believe that recombinant antibodies fulfil these requirements.

We have produced recombinant antibodies to biological warfare agents and simulants using an M13 phage-display system. By using large libraries, many specific antibodies were selected within a few weeks. By modifying the methods of biopanning used we selected for highly specific antibodies that do not react with closely related, non-infectious species of bacteria. We have used the same system to produce antibodies to smaller ligands and haptens. Once useful antibodies have been found they can be produced quickly and cheaply in simple bacterial fermentations. We will also describe ways that we are improving these antibodies by random and directed mutagenesis to improve their utility in biosensors, and show examples of recombinant antibodies giving real time detection on biosensor surfaces.

Process development in antibody purification

Kieran O'Donovan

Protherics UK Ltd

This seminar will discuss the objectives and deliverables of process development within a biopharmaceutical environment. Key to the development of any process is the definition of the edges of failure and outer limit validation which can be determined through the use of scaled down models and multi-factorial experimental design. Through the use of these techniques we can see how process validation can be performed concurrently with process development.

Additionally, due to the nature of antibody production there will also be an introduction into the issues surrounding viral clearance/inactivation and precautions that can be taken.

Process intensification will also be covered suggestion various strategies that can be employed to increase scale and/or profitability of any process.

Therapeutic antibody fragments: expression, purification and function

A. Neil C. Weir

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The development of humanised and human antibody variable domains has allowed therapeutic antibodies to address chronic indications where repeat dosing is now possible due to the reduced immunogenicity of these entities. To complement the above technologies, expression and purification technologies have been developed to supply the large quantities of material needed to support repeat dosing in comparatively common chronic indications such as Rheumatoid Arthritis. Where the proposed mechanism of the antibody based drug is as an antagonist of, for example, a ligand receptor interaction, a fragment, including the variable domain of the antibody, may be used as an alternative to, or in preference to, the intact immunoglobulin G structure. The use of a Fab fragment opens up a range of possibilities for prokaryotic expression of this protein bringing advantages in scale and duration of fermentation. However, since much of the effort and cost associated with the production of an antibody based drug is associated with the recovery and purification stages, improving fermentation efficiency alone is not sufficient and complementary, scalable, downstream processing systems have been developed to recover therapeutic grade material. This presentation will address innovations in the use, expression and purification of Fab based therapeutic entities.

Current developments in human plasma processing

M. Bulmer

Bio Products Laboratory, Elstree

Abstract not received

Microbial Infection Group session

Fundamental and applied aspects of bacterial toxins

Mechanisms of action of clostridial cytotoxins in disease

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Several species of the genus clostridium produce cytotoxins. *Clostridium botulinum* types C and D, *C. perfringens, C. spiroforme* and *C. difficile* synthesize ADP-ribosylating toxins, which modify actin by ADP-ribosylation. Another group of clostridial ADP-ribosyltransferases (e.g., C3 exoenzymes from *C. botulinum* and from *C. limosum*) modify the small GTPases RhoA, B and C, which are switches in numerous cellular processes. Rho GTPases are also the eukaryotic substrates for large clostridial cytotoxins

Members of the family of large clostridial cytotoxins (LCT) are *C. difficile* toxin A and B, the lethal and hemorrhagic toxins from *C. sordellii*, and the alpha-toxin from *C. novyi*. Of particular medical importance are *C. difficile* toxins A and B, which cause antibiotic-associated diarrhoea and pseudomembranous colitis. All these toxins are single chain proteins and have masses of 250 to 308 kDa. The AB toxins have the catalytic domain at the N-terminus and a putative receptor-binding domain at the C-terminus. After receptor binding, LCT are endocytosed. Because bafilomycin blocks toxin effects, it is suggested that cytosolic up-take occurs from early endosomes. Recent results indicate that only the catalytic part of toxin B is translocated into the cytosol.

LCT modify Rho GTPases (Rho, Rac and Cdc42) at threonine 35/37 by mono-O-glucosylation using UDP-Glucose or UDP-GlcNAc (*C. novyi* alpha-toxin) as second substrates. The various toxins somehow differ in their protein substrate specificity. For example toxins A and B appear to modify all Rho proteins, whereas the lethal toxin from *C. sordellii* modifies RhoA, Cdc42 and also Ras proteins but not Rac. Modification of Rho proteins by glucosylation inhibits the interaction of the eukaryotic switch proteins with their effectors. Thereby regulation of the actin cytoskeleton, cell motility, cell cycle control and transcriptional activation of target cells are largely affected.

Studies on anthrax toxin

John Collier

Harvard Medical School, Boston, USA

Anthrax toxin is an ensemble of three monomeric proteins, which are secreted by *Bacillus anthracis* and assemble on the surface of receptor-bearing mammalian cells into toxic complexes. Two of the components [Lethal Factor (LF) and Edema Factor (EF)] are enzymes that modify target substrates within the cytosol. The third [Protective Antigen (PA)] binds to receptors, coordinates assembly of the complexes, and mediates translocation of LF and EF to the cytosol. After binding its receptor PA is proteolytically activated, producing a 63 kDa receptor-bound fragment (PA_{63}), which self-associates to form a ring-shaped heptamer, termed the 'prepore'. The prepore binds a maximum of three copies of EF and/or LF. The complexes are endocyosed and trafficked to an acidic intracellular compartment, where the prepore converts to a pore, mediating translocation of EF and LF. We have identified several sites in the pore forming domain of PA where mutation yields a dominant-negative (DN) phenotype. The DN mutants coassemble with wild-type PA, yielding a mixed prepore that can is unable to undergo the conformational change to the pore and hence cannot translocate EF and LF. Such DN mutants have been shown in cell culture and in rats to be potent antitoxins, while retaining their immunogenic properties. Hence they may be useful both as therapeutic and prophylactic agents for anthrax.

Development of an oral anthrax vaccine: delivery of the *Bacillus anthracis* protective antigen using attenuated *Salmonella*

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The protective antigen (PA) is the central component of the tripartite anthrax toxin secreted by *Bacillus anthracis*. Furthermore, PA is the principle protective immunogen in the current licenced US and UK human vaccines. Since these vaccines are delivered by injection, there is a requirement to develop an orally delivered anthrax vaccine. *Salmonella*-based vaccines potentially offer the advantages of being orally delivered to stimulate long-lived cellular and humoral immune responses following one or two doses. Thus, *Salmonella* expressing PA is being considered as a candidate orally delivered anthrax vaccine. In a previous study, we demonstrated that *Salmonella enterica* serovar Typhimurium exporting PA via the *Escherichia coli* haemolysin (Hly) export system resulted in PA-specific IgG responses in immunised mice and protection against anthrax spore challenge (Garmory *et al.*, 2003). In order to further develop the *Salmonella*-based anthrax vaccine towards clinical trials, we have evaluated the use of attenuated *S. enterica* serovar Typhi vaccine strains BRD948 and Ty21a for the expression and export of PA.

Insight into the variation in cytolethal distending toxin activity between strains of *Campylobacter jejuni* using an immunological and genetic approach

Manal Abuoun, G. Manning, S.A. Cawthraw & D.G. Newell

Veterinary Laboratories Agency, Addlestone

The majority of *C. jejuni* strains express active cytolethal distending toxin (CDT) and possess the three highly conserved genes, *cdt*A, *cdt*B and *cdt*C. Interstrain variation in CDT activity does exist and most *C. jejuni* strains tested to date can be broadly divided into one of two groups: low or high toxin producers. In this study we investigated the basis for this variation in CDT activity at the transcript level using reporter systems. A number of human and poultry isolates have been identified that do not express active CDT and previous investigations in our laboratory identified the genetic basis for this CDT-negativity as either a 667-bp deletion between *cdtA* and *cdtB* or a number of non-synonymous amino acid substitutions occurring in CdtB. Using *in vitro* toxin neutralisation assays we have also demonstrated that CDT is expressed during human infection and is antigenic and that this observed neutralisation of CDT activity, with a range of antisera, occurs in a strain dependent manner. We report the use of immunohistochemistry to identify the point at which these antibodies have their neutralisation effect.

The fail-safe mechanisms of listeriolysin O

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The cell biology of *Listeria monocytogenes* intracellular growth and cell-to-cell spread are well-documented. One of the primary determinants of *L. monocytogenes* pathogenesis is a member of the cholesterol-binding family of pore-forming toxins, listeriolysin O (LLO). LLO is largely responsible for mediating lysis of the host cell vacuole that results upon internalization. We have previously shown that perfringolysin O (PFO) expressed by *L. monocytogenes* can also mediate escape from a vacuole, but subsequently resulted in toxicity to the infected host cell. We have now investigated features of LLO that are distinct from PFO and contribute to pathogenesis. We have identified at least three separate mechanisms that appear to act together to compartmentalize LLO activity to an acidic phagosome and consequently prevent damage to infected host cells: (1) An acidic pH optimum. Mutants with an altered pH optimum are less virulent and damage host cells; (2) LLO is a target of host phosphorylation, perhaps leading to degradation of LLO; and (3) Contains a region within the coding sequence that acts to control LLO translation. Individual mutations have relatively mild phenotypes, but double mutations result in strains that are 4-logs less virulent and extremely toxic to infected cells.

Characterisation of a novel form of pneumolysin from a clinical pneumococcal isolate

Johanna Jefferies[1], **Lea-Ann Kirkham**[1], Yu Jing[1], Andrew Smith[2], Stuart Clarke[1,3] & Tim Mitchell[1]

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Pneumolysin (PLY) is a highly conserved virulence factor of *Streptococcus pneumoniae*, belonging to the family of pore forming toxins known as Cholesterol-Dependent Cytolysins (CDCs). Members of the CDC family possess a conserved region near the C' terminus and mutations created within this area are known to significantly reduce cytotoxicity. PLY is required for full invasive disease and has a range of detrimental effects on the host during infection.

By screening the *ply* gene from 250 clinical isolates of *S. pneumoniae* we discovered an isolate with an eight amino acid repeat upstream from the conserved region. This mutant *ply* allele was found in a serotype 1 isolate. We have cloned and expressed this gene in *Escherichia coli* and purified the toxin in order to characterise its activity and compare the biological properties of this naturally occurring mutant to wildtype toxin.

This work was funded by the Chief Scientist Office of the Scottish Executive.

Role of pneumolysin in pneumococcal disease

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The pneumococcus is an important human pathogen that colonises the upper respiratory tract, eventually leading to diseases of high morbidity and mortality such as pneumonia, septicaemia and meningitis. A key virulence factor in these events is the pneumococcal toxin pneumolysin, which is a 53 kDa protein produced by virtually all clinical isolates of the pneumococcus. Pneumolysin is known to cause a variety of proinflammatory and cytotoxic activities, which significantly contribute to the pathogenesis of invasive pneumococcal disease. An important activity of pneumolysin is its interaction with components of the cellular and humoral immune response. These interactions will be discussed in detail. In models of *in vivo* infection, virulence of pneumolysin-deficient pneumococci are significantly reduced, with lower growth of bacteria in the lungs and reduced development of cellular inflammation, when compared to infections with pneumolysin sufficient pneumococci. The absence of pneumolysin is associated with a significant delay and lower severity of pulmonary inflammation suggesting that pneumolysin is required for successful bacterial virulence. The focus of this presentation will be to discuss in detail several important aspects of host innate immunity to pneumococcal infections *in vivo*, and the role of pneumolysin in these events.

Function and structure of *Photorhabdus* toxins

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Photorhabdus luminescens is a motile Gram-negative insect pathogen that forms a mutualistic symbiosis with entomopathogenic nematodes of the family Heterorhabditidae. Numerous factors have been implicated in the anti-insect virulence of *Photorhabdus* and recent sample sequencing of W14 strain and sequencing of the TT01 genome revealed a plethora of candidate virulence factors. Among these, the toxin complex (Tc) genes form very large (1 megaDalton) complexes of unknown subunit stoichiometry. We have recently shown that the presence of three open reading frames *tcdA*, *tcdB* and a *tccC*-like gene is necessary to reproduce high levels of oral toxicity to *Manduca sexta* when expression in *E. coli* is used. We have also expressed and purified some of the Tc subunits for structural and functional studies.

Another *Photorhabdus* toxin, Mcf (makes caterpillars floppy), causes shedding of the insect midgut epithelium and destructive blebbing of hemocytes suggesting it may trigger apoptosis. Transfection of cells with constructs expressing the N-terminal 1280 amino acids of Mcf, as a fusion with Myc, triggered cell destruction. These and other results confirm that Mcf induces apoptosis but the precise intracellular pathway remains obscure.

An investigation of endonuclease colicin translocation using Surface Plasmon Resonance

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Enzymatic colicins are a family of multidomain bacterial toxins, which kill *Escherichia coli* cells via their nuclease activity. Their cytotoxicity can be inhibited by cognate and non-cognate immunity proteins. The nuclease domain (RNase or DNase) of the colicin must be translocated across both outer and inner bacterial membranes in order to degrade RNA or DNA in the cytoplasm and thus kill the cell. This translocation requires interactions between the N-terminal domain of the colicin and a series of membrane bound and periplasmic proteins called the Tol system (TolB, R, A and Q). We have used Surface Plasmon Resonance to investigate interactions between endonuclease colicin E9 and TolB. Mutation of single residues near the N-terminus of colicin E9 markedly decreases binding to TolB compared to wild-type. The presence of a cognate

or non-cognate immunity protein also reduces binding of the colicin to TolB and this reduction in binding is dependent on the affinity of the immunity protein for the DNase domain of the colicin.

The discovery of the anthrax toxin (Special Lecture)

H. Smith

University of Birmingham

Anthrax kills many animal species, and was used to prove Koch's Postulates in 1876. Soon afterwards, the classical bacterial toxins from other species were produced *in vitro*, but until 1950 a lethal toxin had not been demonstrated in either anthrax bacilli or culture filtrates. The cause of death had been an enigma for 70 years. During the 1950's, a toxin was recognized by examining bacteria and their products obtained from guinea pigs dying of anthrax. The toxin was in their plasma and was shown to contain 2 components. It was then produced *in vitro* and a third component recognized. This work reawakened interest in bacterial toxins and showed that toxins could be multicomponent. It demonstrated for the first time that previously unknown determinants of bacterial pathogenicity could be discovered by examining organisms grown *in vivo*, now a vogue subject in microbiology.

Tetanus toxin and its journey in motor neurons

G. Schiavo

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Axonal retrograde transport is essential for neuronal survival. Pathogens and virulence factors, such as tetanus (TeNT) and botulinum (BoNT) neurotoxins, exploit this pathway to reach their targets within the nervous system. At the neuromuscular junction, these neurotoxins bind with high affinity to lipid microdomains. After internalisation, BoNTs remain at the neuromuscular junction, whereas TeNT is transported to the soma, where enters inhibitory interneurons.

To investigate this differential trafficking in motor neurons, we set up a transport assay using a TeNT fragment (TeNT H_C) as a probe. TeNT H_C is rapidly endocytosed and transported in vesicular carriers of distinct size and speed, which lack typical endocytic markers and are not acidified during axonal transport. Retrograde transport of TeNT H_C in motor neurons requires the integrity of microtubules and actin cytoskeleton and relies on the non-redundant activities of several molecular motors, including dynein and myosinVa. This finding suggests that coordination between these molecules is needed for fast axonal retrograde transport in motor neurons. NGF and TeNT H_C share the same retrograde carriers, which contain the low-affinity neurotrophin receptor $p75^{NTR}$. This assay provides the basis for a better understanding of TeNT trafficking in neurons and represents an ideal tool for the characterisation of the retrograde transport machinery.

Bacterial toxins and cancer: is there a link?

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As the molecular mechanisms of cancer are becoming better understood, it is recognised that many different factors can contribute to oncogenesis. The possibility of a link between bacteria and cancer was controversial for a long time. The strong association of *Helicobacter pylori* with gastric adenocarcinoma, coupled with the known association between certain viruses and increased cancer risk, showed that cancer could have an infectious aetiology. More recently, other chronic bacterial infections have also been shown to predispose to cancer. However, the molecular mechanisms involved in this process are not well understood.

Many bacterial protein toxins that act intracellularly are now known to modify crucial targets in the cell that regulate signalling, and thus to perturb their normal regulation. Many of these signalling proteins are either mutated or differentially regulated during carcinogenesis and so it is possible that some bacterial toxins could act directly to promote cancer. Most notably, the *Pasteurella multocida* toxin acts as a mitogen to stimulate several signalling pathways implicated in oncogenesis. Other toxins also stimulate signalling molecules associated with an increased likelihood of cancer, although the link between bacterial toxins and cancer has not yet been proven.

New toxins and novel vaccine antigens identified by genome analysis of *Neisseria meningitidis*

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The Genomic Era represents a new challenge for the identification of bacterial factors involved in virulence. Thanks to the growing number of sequenced bacterial genomes, it is possible to proceed by homology criteria to predict novel virulence factors in different micro-organisms. Here we will describe a novel ADP-ribosyltransferase and a novel adhesin-invasin identified by genome analysis of the meningococcus B MC58 strain.

ADP-ribosyltransferases constitute a class of functionally conserved enzymes, which display toxic activity in a variety of bacterial pathogens. We have developed a novel pattern-based computational approach, which, flanked by secondary structure prediction tools, has allowed the identification of NarE, a new putative ADPRTs. NarE shows structural homologies with *Escherichia coli* heat-labile enterotoxin (LT) and cholera toxin (CT). The predicted catalytic residues are localized on secondary structure elements compatible with the formation of a properly folded active site cleft. NarE has been shown to possess the predicted enzymatic activity.

NadA, is a surface-exposed antigen of *Neisseria meningitidis* able to form high molecular weight oligomers. The protein organization closely resembles that of YadA of enteropathogenic *Yersinia*. NadA is able to promote adhesion to and invasion into Chang epithelial cells.

The activities of NarE and NadA suggest their possible contribution in the virulence of meningococcus.

Analysis of gene expression in CD4 T-cells from mice immunised intranasally with heat labile toxin from *Escherichia coli*

R.E. Smith, P. Herzyk, C. Young, M. Pizza, R. Rappuoli, G. Hoyne & **G. Douce**

Division of Infection and Immunity, University of Glasgow

Activation of immune responses at mucosal surfaces is important in the protection of the host from infection by pathogens. However, induction of mucosal immune responses to soluble antigens is problematic. This is because soluble antigens often fail to stimulate productive immune responses favouring instead the generation of tolerance. Using a murine model of mucosal tolerance we have demonstrated that intranasal (i.n.) delivery of an immunodominant peptide epitope (110–131) from the house dust mite allergen Der p 1 can lead to the development of long lasting, antigen-specific tolerance. I.N. delivery of the peptide leads to transient activation of CD4+ T cells that gives rise to the generation of antigen-specific CD4+ T regulatory (Tr) cells. However, if the peptide tolerogen is co-administered with the mucosal adjuvant, heat labile toxin, LT, from

E. coli tolerance induction can be prevented. Using microarray technologies we have attempted to determine whether it is possible to discriminate differences in the pattern of gene expression in CD4+ T cells taken from tolerised or immune animals. This presentation will describe the data generated and speculate on the eukaryotic cell signalling that occurs in response to LT. It will also describe the difficulties encountered and the methods employed to overcome them.

Novel therapeutic uses of botulinum toxin

John Chaddock, Cliff Shone & Keith Foster

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Botulinum neurotoxin (the cause of botulism) is one of the most toxic substances known to man. Produced by a range of *Clostridia* sp., the native neurotoxins block the release of chemical signals from motor nerve endings so inducing a flaccid muscular paralysis. In the mid-1970's, clinicians identified that this highly specific and potent mechanism of action could be harnessed for the treatment of conditions that result from hyperactive muscle contraction. This work has led to the widespread use of botulinum neurotoxin as first-line treatment for dystonias and a variety of other muscle spasms. The neurotoxins have evolved unique biological properties that make them both potent neurotoxins and effective medicines. By engineering these natural molecules to interact with a wider scope of cell types, the potential to develop novel medicines based on botulinum neurotoxins for a range of chronic diseases is being explored. Data obtained in pursuit of developing novel analgesics have indicated that such engineered agents can be specifically targeted to cells of therapeutic value, are effective in inhibiting the transmitter release processes of the target cell, and maintain activity for a prolonged period, lasting for many weeks. These exciting research results and their therapeutic potential will be described.

Physiology, Biochemistry & Molecular Genetics Group session

RNA–protein interactions in the regulation of gene expression

mRNA interferases and cell death

M. Inouye

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mRNA interferases (MIases) are a novel class of enzymes, which function as highly sequence-specific endoribonucleases targeting cellular mRNAs. MazF has recently been found in our laboratory to be such an MIase encoded by the *E. coli* genome that causes programmed cell death under certain stress conditions. When MazF expression is induced, cellular mRNAs are cleaved at ACA sequences resulting in complete inhibition of protein synthesis, that leads to cell growth arrest and eventually cell death. MazF homologues are widely found in most bacteria, and I will present data demonstrating that these MazF homologues also function as MIases having different sequent specificities. These MazF homologues include PemK (Kid) from plasmid R100 and MazF-mt1 from Mycobacterium tuberculosis. I would like to discuss the possible roles of MIases in bacterial physiology and population control. In addition, as MIases function both in prokaryotes and eukaryotes, they can be used as a regulatable suicidal protein applicable for gene therapy. Furthermore, the use of MIases enables us to develop a number of new biotechnological methods, which will also be discussed in this talk.

Regulating transcription attenuation in the *B. subtilis trp* operon by an RNA-binding protein: structural and mechanistic issues

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The *trp* RNA-binding Attenuation Protein (TRAP) regulates expression of genes involved in tryptophan metabolism in *Bacillus subtilis* by transcription attenuation and translation control mechanisms. TRAP is composed of 11 identical subunits arranged in a ring. Tryptophan-activated TRAP binds to several RNA sites consisting of up to 11 GAG or UAG repeats separated by non-conserved spacer nucleotides. We have used nuclease footprinting to characterize binding of TRAP to several binding sites composed of 11 GAG repeats. The affinities of TRAP for individual repeats within the 11 repeat binding sites do not vary significantly. In contrast, association rate constants for TRAP binding were fastest for repeats at the 5'end and slower towards the 3'end of all binding sites tested. This observation suggests that binding initiates at 5'end of the binding site followed by wrapping the RNA around TRAP in the 5' to 3' direction. We suggest that this mechanism is important for TRAP-mediated transcription attenuation control of the *trp* operon. In addition, accumulation of uncharged $tRNA^{trp}$ induces synthesis of Anti-TRAP (AT). AT modulates expression of the *trp* genes indirectly by binding to TRAP and preventing formation of TRAP/RNA complex. AT exists in a reversible equilibrium between trimer and dodecamer.

E. coli DbpA – a sequence-specific RNA helicase

O. Uhlenbeck

Northwestern University, Illinois, USA

Abstract not received

Ribosome synthesis and the cell-cycle: integration or opportunism?

Marlene Oeffinger[1], Alessandro Fatica[2] & **David Tollervey**[3]

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Changes in nucleolar structure have long been recognized as a marker for malignant transformation of human cells. The nucleolus is the site of ribosome synthesis, the rate of which is tightly coupled to the cell growth rate. However, in many cancers growth is not increased, but cells fail to undergo appropriate cell-cycle arrest and/or apoptosis. This suggests that changes in the nucleolus have significance in cell transformation beyond reflecting alterations in ribosome synthesis rates.

In yeast, several proteins are implicated in both ribosome synthesis and cell-cycle progression. Net1p was previously shown to regulate mitotic exit via sequestration and release of the phosphatase Cdc14p. We have now shown that depletion of a newly characterized ribosome synthesis factor, Rrp14p, blocks mitotic progression and causes striking defects in positioning of the mitotic spindle. Rrp14p was previously reported to interact with proteins involved in bud site selection and cell morphology, suggesting a direct function. Cells depleted for another ribosome synthesis factor, Nop15p, exit from mitosis correctly, but fail to assemble a contractile actin ring at the bud neck and therefore fail to undergo cytokinesis. Nop15p is predominately nucleolar during interphase but is released into the nucleoplasm during mitosis, suggesting that its role in cytokinesis may be distinct from its function in ribosome synthesis. Mutations in other ribosome synthesis factors are reported lead to defects in bud site selection or the size of the cell at division, and several are reported to be associated with DNA replication complexes.

There appear to be connections between nucleolar proteins and multiple steps in the cell cycle. An outstanding question is whether these proteins have been recruited to distinct secondary functions, or act to integrate ribosome synthesis with cell-cycle progression?

Lsm proteins and RNA processing in *Saccharomyces cerevisiae*

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Our focus is on studies of nuclear pre-mRNA splicing in the budding yeast *Saccharomyces cerevisiae*. In addition, we are interested in how splicing and other pathways of RNA metabolism are linked in order to coordinate these processes.

The Lsm (Like Sm) proteins are a conserved group of small proteins that are structurally related to the Sm proteins. Their homology to canonical Sm proteins, as well as electron microscopy and physical interaction data, suggest that the Lsm proteins form seven-member rings that interact with RNA. A complex of seven Lsm proteins, Lsm1–7, is involved in mRNA degradation in the cytoplasm. Another heptameric Lsm complex, Lsm2–8, exists in the nucleus and is mostly found associated with the spliceosomal U6 snRNA. This complex is necessary for efficient nuclear pre-mRNA splicing and for recycling U6 snRNPs. In addition, Lsm proteins are involved in

the degradation of pre-mRNAs and in the processing of pre-tRNAs, pre-rRNAs and pre-snoRNAs in the nucleus. As well as studying the functions of the Lsm proteins, we are investigating how the different Lsm protein complexes form and what determines their cellular localisation.

Structural and functional studies of the eukaryotic translation initiation pathway

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Translation initiation involves assembly of the 43S preinitiation complex, recruitment of this complex to the mRNA (via the 5'end), scanning, recognition of the reading frame start codon, and 60S ribosomal subunit joining. Recruitment of eukaryotic ribosomes to the vast majority of mRNAs is mediated by the cap-binding complex eIF4F, which has at its core the cap-binding protein eIF4E and the much larger multidomain factor eIF4G. A number of the initiation factors (eIF3, eIF1, Met-tRNA$_i^{Met}$.eIF2.GTP, eIF5) form a large Multi-Factor Complex (MFC) which is likely to be a major intermediate on the pathway towards the assembly of the 43S ribosomal complex. The eIF4E-eIF4G interaction is critical, since it tethers the MFC, and thus the 40S ribosomal subunit, to the 5'end of the mRNA.

This presentation will review recent work, including NMR and cryo-EM studies, that shed light on the eukaryotic 43S assembly pathway.

Hfq: A bacterial Sm-like protein that play key roles in riboregulation

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The bacterial Hfq protein was originally discovered in *E. coli* in 1968 as a host factor (HF-I) required for bacteriophage Qβ RNA replication. During the last decade Hfq has emerged as a global post-transcriptional regulator which controls the expression of many proteins by affecting mRNA translation and/or stability (for a review, see 1). The mechanism of the effect of Hfq on mRNA translation appears to involve its influence on the interaction of small regulatory RNAs with target mRNAs. Indeed, several studies have established that Hfq, which has a binding preference for A/U-rich sequences, binds strongly to many chromosomally-encoded riboregulators that act by complementary pairing with their targets, and is required for the activity of many of them. Part of this requirement might be the result of the stabilization of the riboregulators by Hfq. However, Hfq might also function independently of effects on sRNA stability by facilitating RNA-RNA interaction. Insight into this latter function of Hfq has come from studies of the OxyS RNA and Spot 42 RNA and it has been proposed that Hfq acts as a general cofactor for antisense RNAs that rely on short stretches of base pairing (2–4). The ability of Hfq to mediate RNA-RNA interactions and the identification of an Sm-1 motif by sequence analyses suggested that Hfq was the bacterial equivalent of the eukaryotic Sm proteins (2, 4). Characterisation of the secondary structure of Hfq by circular dichroism supported this postulate as did electron microscopy studies, which revealed a doughnut-shaped oligomeric molecule. The full extent of the structural homology between Hfq and Sm and Lsm proteins has now been realised by the crystal structure determination of the *S. aureus* Hfq (5) and confirmed very recently by the structure of a C-terminally truncated Hfq from *E. coli* (6). In addition, insight into the RNA binding mechanism of Hfq, specifically its preference for uridine and adenosine tracts, has been gained by a Hfq-uridine-rich oligonucleotide complex (5). Here I will describe the novel findings about Hfq, its distribution among prokaryotes and briefly discuss the action of a few novel riboregulators that have provided explanations for previously mysterious regulatory effects.

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CsrA and its small RNA antagonists, CsrB and CsrC

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The carbon storage regulatory system (Csr; also called Rsm, repressor of stationary phase metabolism) utilizes a 61 amino acid RNA binding protein, CsrA, to regulate translation and mRNA stability. In so doing, it governs cell physiology and metabolism on a broad scale. In general, CsrA represses genes and processes that are associated with the stationary phase of growth, while it activates gene expression associated with exponential phase. CsrA also binds to two small untranslated RNAs, CsrB and CsrC. In the case of CsrB, this binding has been shown to result in the formation of a large RNP complex, composed of ~18 subunits of CsrA and one CsrB transcript. CsrB and CsrC are antagonists of CsrA, and binding to CsrA reversibly inhibits its activity. This provides a homeostatic mechanism for maintaining tight control of CsrA activity, since CsrA itself indirectly activates transcription of the *csrB* and *csrC* genes. This presentation will address the molecular mechanisms, signaling pathways and physiological functions of the Csr system.

Rescue of stalling ribosomes

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When bacterial cells are exposed to amino acid starvation in down-shift situations, missing enzymes must be rapidly synthesized for fast recovery of exponential growth in the new situation. When amino acids are limiting, ribosomes stall at codons cognate to the most limiting amino acid.

The mechanisms of action of the bacteriotoxin RelE and tmRNA are discussed, along with their putative roles for fast growth-recovery after down-shifts. The finding that RelE cleaves mRNAs in the A site of the ribosome at selected sense or stop codons is recapitulated. It is demonstrated that the first step in trans-translation by tmRNA *requires* truncated mRNAs, and the reasons for this are discussed.

Recent *in vivo* data suggest that there exist multiple mechanisms of mRNA truncation in response to ribosome pausing along with mechanisms for rescuing pausing ribosomes other than the tmRNA pathway. These findings are discussed in the perspective of bacterial adaptation.

Post-transcriptional regulation by bacterial aconitases

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Mammalian cells possess two aconitases; a mitochondrial aconitase that functions in the citric acid cycle, and a bifunctional cytoplasmic aconitase. During periods of iron sufficiency bifunctional aconitases acquire enzymic activity by assembling [4Fe–4S] clusters, but during iron-starvation, or exposure to oxidative stress, these iron-sulphur clusters are disassembled, catalytic activity is lost and the resulting apo-proteins bind to specific mRNAs to regulate their translation. *Escherichia coli* has two aconitases (AcnA and AcnB) with different physiological roles (Jordan *et al.*, 1999 *Biochemical Journal* **344,** 739–746). Recently it ahs been sown that both *E. coli* aconitases (Tang and Guest, 1999 *Microbiology* **145,** 3069–3079) and the aconitase protein of *Bacillus subtilis* (Alen and Sonenshein, 1999, *Proceedings of the National Academy of Sciences USA*, **96,** 10412–10417) are also involved in translational regulation. The structure of AcnB indicates that the catalytic/RNA-binding region is similar to that of the eukaryotic aconitases suggesting that the same determinants are used for RNA-recognition (Williams *et al.*, 2002 *Nature Structural Biology* **9,** 447–452). The *E. coli* aconitases are implicated in mediating a rapidly reacting post-transcriptional response to oxidative stress partly by controlling the synthesis of superoxide dismutase (Tang *et al.*, 2002 *Microbiology* **148,** 1027–1037). Furthermore, *Salmonella enterica acnB* mutants display defective binding to macrophages and impaired motility. Analysis of the causes of the latter defect revealed the presence of a regulatory cascade consisting of AcnB, FtsH, σ^{32}, DnaK and FliC (Tang *et al.*, 2004 *Molecular Microbiology* in press). The involvement of the protease FtsH and the alternative sigma factor σ^{32} suggests that aconitase-mediated post-transcriptional regulation will have far reaching implications in coordinating gene expression by linking central metabolism to oxidative stress, iron-starvation and the heat shock response.

The degradation of mRNA decay intermediates in *E. coli* by the RNA degradosome and poly(A) polymerase

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The *Escherichia coli* RNA degradosome is a multienzyme complex containing RNase E, PNPase, RhlB and enolase. RhlB is a DEAD-box RNA helicase. To elucidate its function *in vivo*, we have disrupted its gene and combined this mutation with mutations in other genes involved in mRNA degradation. Our results show the accumulation of mRNA decay intermediates in a strain in which *rhlB* and *pcnB*, encoding poly(A) polymerase, were disrupted. These enzymes appear to act in separate pathways since full stabilization of the decay intermediates depends on knocking out both activities. This is the first *in vivo* evidence that RhlB is involved in mRNA degradation. Other *in vivo* results show that RhlB and PNPase act together as components of the RNA degradosome demonstrating for the first time that the RNA degradosome is required for the RhlB-PNPase pathway. Furthermore, the poly(A)-dependent pathway appears to involve either free PNPase and/or another ribonuclease that has not yet been identified.

Regulation of mRNA turnover in yeast

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Control of mRNA turnover is often coupled to the mRNA's ability to translate, thus providing quality control networks for mRNAs that are aberrant and/or no longer functional. In yeast, a key step in decay appears to be deadenylation-dependent decapping, which is followed by 5' to 3' exonucleolytic digestion. Before mRNAs are decapped, however, they must first undergo a critical transition from a translationally active state, into an inactive or repressed state. Exiting the translational pool represents a critical node in the control of turnover and is influenced by several interwoven aspects of mRNA metabolism. These tightly coupled aspects include, the translational efficiency and deadenylation rate of the mRNA, the presence of *cis*-acting sequences, and interactions with *trans*-acting factors. Of the known factors which bind to mRNAs and stimulate decapping, some may do so by helping to promote translational repression rather than by directly acting on mRNA decapping *per se*. Recent studies of such factors suggest fundamental aspects of cytoplasmic mRNA physiology that connect decapping, translation, and storage of mRNA.

Systematics & Evolution Group / Clinical Microbiology Group joint session

Clinical diagnostics: current applications and future prospects

The emerging brave new world of microbiology

S.P. Borriello

Health Protection Agency, London

A revolution is occurring in nucleic acid analysis, bioinformatics, data storage and retrieval, nanotechnology, physics, micro-electronics, and polymer, solid state and combinatorial chemistry. These seemingly disparate fields are being combined and applied to the detection, identification, and characterisation of pathogens. The once science fiction scenario of taking a drop of blood, urine or saliva and within an hour knowing whether or not a pathogen is present, its type designation and its antimicrobial resistance potential will soon be a reality. The application of mass spectrometry to microbiology (MALDI-ToF and SELDI-ToF) is also set to have a major impact. These developments, particularly with regard to near patient testing, have important implications for the delivery of health care. They will have an impact on primary care, prescribing practice, organisation of pathology laboratories, counselling services, surveillance and epidemiology, and for medico-legal practice. (S.P. Borriello [1999] *BMJ* **319**, 298–301). New molecular methodologies are facilitating real-time strain differentiation for out-break investigations with detection of antimicrobial susceptibility potential. Further, new light is being shed on epidemiology of infectious diseases for established diseases, and new pathogens are being uncovered.

Rapid identification and antimicrobial sensitivity testing in clinical microbiology

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Infectious diseases are the second leading cause of death worldwide, behind cardiovascular disease, and the third leading cause of death in the USA. Despite the global human and financial cost of infectious diseases the majority of routine hospital based tests for determining the presence of disease-causing bacteria still use labour-intensive techniques developed by Pasteur 130 years ago, which take 2–3 days to obtain a result. This protracted testing process means that current microbiology practice rarely affects immediate patient management but is used to educate clinician's future diagnosis and treatment options.

In the last 5–10 years improvements in the clinical laboratory have focused on standardising laboratory practice and improving laboratory efficiency by automating current laboratory practice. These changes have improved laboratory workflow, data consistency, and helped laboratories manage staff recruitment problems.

In our efforts to improve laboratory efficiency little thought has been given to the clinical value of diagnostic microbiology, with a recent laboratory audit identifying 46% of microbiology tests as inappropriate.

As we consider the potential of new technologies to help in the laboratory diagnosis of infectious diseases it is important that these new technologies are viewed in the context of their contribution to improving patient management and public health and we question the conventional paradigm. To quote Einstein, '*the problems we face cannot be solved by the same level of thinking that created them*'.

Genotypic characterisation of *Neisseria meningitidis* using a CustomSeq™ microarray

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Neisseria meningitidis (meningococcus) is a major cause of meningitis and septicaemia and can cause endemic and epidemic disease. Sequence typing is currently the 'gold standard' for meningococcal typing and is used in combination with phenotypic methods for strain characterisation. However, there are limitations to both methodologies. DNA microarrays permit the rapid analysis of a large amount of genetic information. CustomSeq™ is a high-throughput, high-density resequencing microarray. The methodology is based on a 25-nucleotide probe 'tiling-strategy' where four probes are used to identify the central nucleotide of the sequence. This approach can be used to sequencially interrogate a given sequence and also to identify Single Nucleotide Polymorphisms (SNPs). The microarray in this study was designed to include all known variants for genotyping (*porB*) and genosubtyping (*porA*) and also sequences to predict genogroup (*ctrA*, *siaD*). In total, 75 meningococcal isolates with diverse serogroups, serotypes, serosubtypes and genosubtypes were tested. The array successfully predicted serogroup, serotype and serosubtype. There was 100% concordence with previous genosubtype data (*porA*) and all samples were newly genotyped for *porB*.

In conclusion, this is the first application of CustomSeq™ to microbial typing. The technology can discriminate to SNP level. This prototype scheme could be applied to other organisms where extensive sequence data is required for typing.

Diagnosis of central venous catheter associated bacterial infection using 16S rDNA detection

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Many central venous catheters (CVC) are removed unnecessarily because current diagnostic methods are unreliable. We have evaluated quantitative real time assays using primers and probes targeted at 16S rDNA to measure levels of bacterial DNA in blood samples drawn from the CVC of both adults receiving intravenous nutrition and children undergoing treatment for cancer. Bacterial DNA concentrations in adults receiving intravenous nutrition were raised in 16/16 blood samples taken during episodes of probable bacterial CVC associated infection and in 4/29 episodes where bacterial CVC infection was unlikely. Receiver operating curves were used to calculate the optimum threshold values for quantitative bacterial DNA levels. We are developing an economic evaluation of this test for a population of children with CVCs and undergoing treatment for cancer. In preliminary results in children being treated for cancer high levels of bacterial DNA were found in the blood in association with conditions other than CVC associated infection. These included severe mucositis and bacteraemia. Threshold values for diagnosing CVC associated infection in an immunocompromised population are higher than in the immunocompetent.

The impact of quantitative real-time PCR in diagnostic microbiology

M. Guiver

HPA NorthWest, Manchester

The exquisite sensitivity and specificity of nucleic acid amplification techniques has found wide application in clinical microbiology. While a qualitative result may be adequate in some situations for the demonstration of a pathogen, frequently it is useful to determine the numbers of organisms present in the original sample in monitoring therapy or to assess the pathogen load in discriminating latent and active infection.

Quantification by conventional PCR methods using end-point product analysis is frequently limited in dynamic range and often requires further dilution of sample or PCR product.

Quantitative real-time PCR allows the amplification process to be monitored during thermal-cycling and it is in the early stages of amplification when the amount of generated product is directly proportional to the amount of input nucleic acid. Real-time PCR is the most accurate and reliable approach to quantitative PCR and has also been of importance in gene expression studies. Real-time PCR also confers additional benefits such as closed tube detection and rapid processing times, important in providing a routine diagnostic service.

There are a variety of real-time nucleic acid amplification methods and many have now been developed commercially.

A review of the approaches to real-time PCR detection will be presented and some of the applications and the impact in patient management.

Moving from observational toward computational methods in bacterial identification

S. Fischer

NIH Warren Grant Magnuson Clinical Center, Bethesda, USA

In the US model the clinical microbiology laboratory performs: (1) detection and identification of infectious agents from patient materials; (2) quantification of organisms in individual samples; (3) predictions of treatment success for a given drug-bug-site combination; and (4) limited epidemiologic studies to aid in infection control.

To do these activities efficiently a clinical microbiology lab employees a variety of specialized techniques and equipment for sample processing and testing based on applications of knowledge of organism growth requirements, biochemistry, morphology and microscopic staining properties, antigenic properties and host immune responses. In addition, laboratories employ methods for tracking individual samples and for attributing laboratory information obtained to the right specimen and patient.

The successful application of these approaches for providing useful information is assessed by measures of sensitivity, specificity, positive predictive values for disease, negative predictive values for disease and turn-around-times.

Other real-world considerations affecting the value of clinical microbiology include: costs of operating the laboratory; the ability to meet regulatory requirements for quality assurance, data management and confidentiality; difficulties in maintaining a cadre of well-trained specialists; and the ability to provide clinical interpretations of laboratory results.

Could a purely molecular approach ever replace traditional methods?

Rapid pathogen detection in biodefence

Martin Pearce

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Battlefield detection of microbial pathogens and diagnosis of infectious disease are key components of the UK Nuclear, Biological and Chemical (NBC) Defence Strategy. The requirement is for rapid, sensitive and specific assays for identification of biological warfare (BW) agents in clinical or environmental samples so that appropriate protective measures may be taken. The Defence Science and Technology Laboratory at Porton Down is researching physical and biochemical methods for the identification of pathogens in the field, including flow-through detection systems capable of real-time pathogen identification. Clinical diagnostic systems are also being developed which enable the movement of diagnostic tests closer to the point of infection. This presentation will describe Dstl research in the areas of real-time and near real-time sensor development including antibody and DNA based detection systems as well as other technologies required to overcome the unique challenges of detection and diagnosis on the battlefield.

Review of rapid approaches to strain typing

T.L. Pitt

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A wide variety of methods are available to microbiologists for the sub-specific division of bacteria and the designation of strain types. Some of these methods have been used for several decades while others have only recently been described. Not all can be termed rapid and they vary considerably in their discrimination and reproducibility. The oldest of rapid methods are the serological and phage typing systems which are still widely used, although often now the preserve of reference laboratories, and are of particular value for grouping isolates from epidemiological incidents and identifying outliers in an outbreak. For fine discrimination, the PCR based systems such as random amplified fragment length polymorphism (RAPD), repetitive (REP)-PCR, and fluorescent fragment length polymorphism (AFLP) generate fingerprints which can be compared either by Pearson correlation or Dice coefficients with the aid of computer software. Variable number tandem repeats (VNTR) are repeated multiple times in a genome sequence and often vary in copy number thus forming polymorphisms that can be detected by PCR using flanking primers. They appear to offer greater discrimination than most other PCR methods and multilocus systems (MLVA) are being applied to a number of genera but particularly for those with extreme genomic conservation. Many rapid DNA macrorestriction digest protocols have been reported but optimal resolution of DNA profiles by pulsed-field gel electrophoresis (PFGE) is often achieved using relatively long running times. Sequence based typing systems such as multilocus sequence typing (MLST) which indexes allelic variation in house keeping genes is rapidly becoming the strain designation standard for a number of bacterial species but the technique is labour intensive. Highly polymorphic regions of specific genes or those under greater selective pressure, which are often more reflective of recent genomic change than the conserved housekeeping genes, are increasingly being targeted as rapid strain typing markers, e.g. *spa* (protein A) gene typing for *Staphylococcus aureus*, and surface expressed proteins and lipopolysaccharide biosynthetic genes for *Neisseria meningitidis*. DNA expression microarrays may have only limited application to rapid strain typing due to evolutionary divergence, expression plasticity and the effect of artefacts such as growth conditions on expression.

Immobilized molecular beacons in rapid pathogen diagnostics

Benjamin L. Miller, Todd D. Krauss, Nicole Crnkovich, Hui Du & Christopher M. Strohsahl

University of Rochester

The development of new easy-to-use and inexpensive methods for the rapid detection of pathogenic organisms is an important 'basic science' goal, and has broad potential applications in the improvement of human health. We have recently described a sensor chip, derived from the 'molecular beacon' solution-phase assay concept, that is able to selectively and sensitively detect target DNA sequences without the need for external sample processing or fluorescence labeling. In this implementation, a DNA hairpin probe is functionalized at one end with a fluorophore, at the other end with a disulfide, and then immobilized on a gold film. Thus, in the absence of the target complementary DNA, the sensor itself quenches the fluorescence of the probe. Binding of the target DNA causes the hairpin to 'unfurl', moving the fluorophore away from the gold surface and preventing fluorescence quenching. In this lecture, I will describe further studies on the sensitivity of this system to single base mismatches, new methods for the identification and design of molecular beacon probe sequences, and the application of this technique to the detection of a variety of bacterial and fungal pathogens (including methicillin-resistant *Staphylococcus aureus* and the common skin pathogen *Trichophyton tonsurans*).

Microarray potential for blood screening and diagnostics

Juraj Petrik

Head, Microbiology R&D, Scottish National Blood Transfusion Service (SNBTS)

Introduction of nucleic acid amplification techniques (NAT) into routine blood screening for viruses has further improved the safety of the blood supply. However, the testing efficiencies for viral targets, blood grouping and bacterial contamination differ significantly, reflecting in part the use of multiple instrumental platforms. Microarray technology has the potential to provide a single testing platform and to significantly simplify blood bank operations.

Numerous design features to be considered in the development of such complex testing system include integration of the sample preparation/concentration step, on-chip target and/or signal amplification, parallel design of protein and nucleic acid-based chips, target selection criteria, etc.

Antibody/antigen arrays represent an obvious diagnostic interest for blood grouping and microbiology testing. A combined chip containing selected blood grouping probes and probes specific for blood borne pathogens is being addressed as the first step in the development of a more complex testing array in a collaborative effort between SNBTS and the University of Edinburgh Genomic Technology and Informatics Centre.

Are there genes associated with invasive pneumococcal disease?

Johanna Jefferies[1], Stuart Clarke[1,2], Andrew Smith[3], Chris Dowson[4] & Tim Mitchell[1]

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Multi Locus Sequence Typing (MLST) is a portable sequence-based typing method that has been used to identify clones and clonal groups by analysis of genetic relationships between housekeeping genes in several bacterial species. In disease causing bacteria it is pertinent to investigate whether virulence factors can be mapped directly to these clonal groups or whether more complex relationships exist when considering genes that may be under strong selection pressure from the host immune system.

We have used MLST to characterise a collection of 250 pneumococcal isolates and have also obtained sequence data from three genes thought to be involved in the virulence of the pneumococcus, *ply*, *hylA* and *nanA* encoding pneumolysin, hyaluronidase and neuraminidaseA respectively. Sequencing of virulence determinants from pathogenic bacteria may lead to the association of certain alleles with disease or to the type of disease an organism may cause. This knowledge will be important in future vaccine design.

Pneumococcal virulence determinants have also been detected in a number of oral streptococci and atypical pneumococci. In this second group of organisms the sequence of *ply* differs from those present in the pneumococci. We suggest that sequence analysis of virulence genes in this way should be developed further for differentiation of pneumococci and closely related organisms.

Identification of bacterial pathogens from pleural fluids using 16S PCR and sequencing

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16S PCR and sequencing is a useful technique for identifying organisms with difficult to interpret biochemical profiles. Using this technique on clinical samples from sterile sites has not been widely employed. 431 pleural fluids from patients with empyema were subjected to 16S PCR and sequencing and a method optimised for identification of pathogenic bacteria from pus.

Of these 431 samples, 287 produced 16S PCR amplimers and 144 were negative. Sequencing of PCR amplimers produced 132 un-readable sequences, 2 likely contaminants and identified 153 likely empyema pathogens. The empyema pathogens identified included 44 *Streptococcus pneumoniae* (29%), *Streptococcus intermedius* (19%), *Staphylococcus aureus* (11%), *Fusobacterium nucleatum* (9%), *Prevotella oris* (7%), *Streptococcus pyogenes* (5%), *Enterococcus faecium* (3%), *Bacteriodes fragilis* (2%), *Streptococcus sanguinis* (1%) and 22 other bacteria (14%).

Unreadable sequences included reactions that showed good but mixed sequences where many peaks had equal intensity for two nucleotides and very weak reactions that are likely to have resulted from poor DNA quality from the original clinical sample. A strategy for investigating the samples that did not produce a readable sequence will be discussed.

Point-of-care diagnostics: HyBeacon™ probes

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HyBeacon probes are fluorescent oligonucleotides that are utilised in real-time PCR assays to rapidly detect and identify nucleic acid sequences. HyBeacons may be employed in numerous diagnostic sectors such as health care, food analysis and defence for applications including genetic diagnosis and pathogen detection. Probes emit considerably more fluorescence when hybridised to complementary target sequences than when single-stranded. Assays measure the stability of HyBeacon hybridisation and employ melt peak analysis to characterise target sequences. Closely related sequences, differing by as little as a single nucleotide, may be discriminated by measuring the melting temperatures (Tm) of probe/target duplexes. A single HyBeacon probe may be employed to simultaneously analyse multiple sequence variants, allowing reliable identification of

homozygous and heterozygous samples or concurrent detection of a target and a positive amplification control. Samples may be analysed directly, without prior purification of nucleic acids, within 20 minutes using rapid real-time PCR instruments such as the GeneDrive. The speed and ease of analyses performed in the GeneDrive will enable the use of HyBeacon probes outside of specialised research facilities, such as in hospitals and doctors surgeries, for point-of-care diagnostic applications.

Too small to see: the promise of the next generation of sensors

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The utility of diagnostic tools for the detection and identification of pathogens is being advanced by the application of the tools and processes used for microfabrication. Miniaturization of individual components coupled to an overall reduction in the size and weight of the instrument will move these diagnostic devices from the central laboratory to the point-of-care environment. Then wide applications will be realized not only in medicine but in food and environmental testing. We have developed micro-scale components to purify nucleic acid from samples and coupled that to an integrated polymerase chain reaction-based detection system. Amplification of target DNA is monitored in real-time using the incorporation of a fluorescent interchelating dye. This purification-amplification chip has been incorporated into the microFLUIDICS DESKTOP, which contains all of the optical detection, reagent delivery and data capture in a single 13" × 12" × 3.5" package. The system has been tested with a variety of microbial targets including *Listeria monocytogenes, Escherichia coli, Bacillus globigii,* and *Staphylococcus aureus.*

The component design of the microFLUIDICS DESKTOP allows for the further miniaturization of the system with the next immediate goal to produce a truly hand-held portable device followed by the development of a distributed sensor system reaching truly micron-scale proportions.

Virus Group sessions

Viruses and signalling

Signalling in adenovirus entry

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Most of the more than 50 human Adenovirus (Ad) serotypes use the Coxsackie virus B Ad receptor (CAR) or the membrane-cofactor CD46 for cell attachment. The former is involved in cell adhesion and the latter is a ubiquitous regulator of complement activation. The species C serotypes Ad2 and Ad5 bind CAR and induce signalling by activating integrin coreceptors. Downstream signals facilitate viral endocytosis through clathrin-coated pits and trigger a nonspecific type of endocytosis, macropinocytosis. Ad-induced macropinocytosis requires integrins, F-actin, protein kinase C and small G-proteins of the Rho family but not the large GTPase dynamin. Additional signals facilitate macropinosomal lysis and the release of particles to the cytosol. Viral DNA is transported within capsids along microtubules, depending on the minus end-directed motor complex dynein/dynactin. Viral activation of integrins and additional signals tilt the motility balance of capsids towards the microtubule-organizing center (MTOC), proximal to the nuclear membrane. The capsids bind to nuclear pore complexes, disintegrate and release their genomes to the nucleoplasm. Their targeting to the nuclear pores is essential for infectivity. Conditions that arrest the incoming particles at the MTOC block genome import into the nucleus and infection. These observations reflect the intricate relationship of pathogens with their hosts.

Regulation of cell proliferation and viral reactivation in Epstein–Barr virus infection and cancer

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Epstein–Barr virus (EBV) infection of human B lymphocytes causes cell proliferation, EBV persisting latently in the cells. Expression of several viral proteins is required for the cell proliferation, including the transcription factor EBNA-2. Target genes activated by EBNA-2 include c-Myc, Runx3 and a PI3 kinase regulatory subunit. Runx3 is transcription factor and a tumour suppressor gene in gastric cancer. Expression of Runx1 (another member of the Runx gene family) and Runx3 correlates with different infection states of EBV. The mutually exclusive expression that is observed for Runx1 and Runx3 is a consequence of regulation of Runx1 expression by Runx3. The PI3 kinase isoforms present in EBV infected cells have been characterised and shown to be required for proliferation of EBV infected B cells.

Reactivation of the viral lytic cycle from latency can be induced in certain Burkitt's lymphoma cell lines such as Akata by cross linking the B cell receptor to simulate activation by antigen. The induction of EBV immediate early, delayed early and late promoters has been reconstituted on plasmids stably transfected into these cells. This system has allowed genetic and biochemical analysis leading to a model for the process of reactivation of this member of the herpesvirus family.

Geminiviruses, cell proliferation and gene expression

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Geminiviruses, small circular ssDNA plant viruses that encode just a few proteins, are excluded from cells with a high proliferative activity, where DNA replication proteins are functionally active. A rich interplay between viral proteins and host factors, aimed at inducing a cellular environment permissive for viral DNA replication and spread, is established. This includes interactions with components of the cell cycle machinery, the cellular DNA replication, the nucleotide metabolism or the plasmodesmata (plant cell-cell communications), among others. Geminivirus proteins are known to interfere with both the G1/S and the G2/M regulators. In the former case, interference with the plant retinoblastoma-related (RBR)/E2F/DP pathway is of major importance. However, the situation is still poorly understood.

We are currently investigating the structural and functional basis for the interaction between the geminivirus early proteins (RepA and Rep) and the RBR/E2F pathway, in particular the ability of viral proteins to release E2F activity. This has been shown to be crucial for the up-regulation of a number of known E2F target genes involved in the G1/S transition as well as for the changes in cellular DNA replication competence. These topics will be discussed in the context of geminivirus-dependent global and E2F-dependent changes in gene expression.

Modulation of B cell receptor signal transduction by Epstein–Barr virus

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Epstein–Barr virus (EBV) establishes latent infections in the majority of the human population. During latency, there is limited viral gene expression. The virus-encoded latent membrane protein 2A (LMP2A) is one of the most consistently detected viral transcripts in latent infections and EBV-associated cancers such as Hodgkin's Disease (HD). The establishment and maintenance of EBV in B lymphocytes (site of EBV latency) requires the exquisite manipulation of normal B cell signaling and function. LMP2A inhibits normal B cell receptor (BCR) signal transduction preventing activation of lytic replication and provides developmental and survival signals to B cells. The goal of our research is to identify specific cellular pathways targeted by LMP2A. We have utilized microarray technology to monitor changes in gene transcription altered by LMP2A. LMP2A expression results in the reduced expression of several transcription factors critical for B cell development resulting in patterns of gene expression similar to that observed in Reed-Sternberg cells of HD. Finally, we are identifying cellular targets of LMP2A that are important for LMP2A function. Our experiments have identified specific cellular pathways that are altered by LMP2A that may provide a basis for the development of therapeutics that may be useful in the treatment of EBV-associated cancers and EBV latent infections.

Influenza virus induced signaling processes: NF-κB, apoptosis and the 'flu

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Activation of the transcription factor NF-κB is a hallmark of infections by viral pathogens including influenza viruses. Since gene expression of many antiviral cytokines is controlled by the factor, the concept emerged that NF-κB is an essential component of the innate antiviral immune response to infections with RNA viruses. In contrast to that common view we surprisingly observed that NF-κB activity is required for efficient influenza virus production. On a molecular level this includes NF-κB-dependent viral induction of the proapoptotic factors TRAIL and Fas/FasL which enhance virus propagation in an auto- and paracrine fashion. Thus, unexpectedly NF-κB acts both proapoptotic and proviral in the context of an influenza virus infection. This implies that apoptosis induction is also beneficial for the virus and indeed influenza virus production was strongly reduced in cells with impaired function or expression of a major apoptotic mediator, caspase 3. Mechanistically, caspase 3 appeared to promote migration of RNPs to the cytoplasm in late stages of infection. Thus, influenza virus has acquired the capability to misuse antiviral defense mechanisms of the cell to support its own replication.

Cell survival signalling in response to rubella virus infection in RK13 cells

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Rubella virus (RV) causes severe congenital defects when contracted during the first trimester of pregnancy. The molecular mechanisms governing the development of these defects are not fully understood. RV-induced apoptosis has been suggested as a mechanism for disruption of organogenesis during congenital infection, since the cytopathic effect induced by RV in a number of susceptible cell lines is due to caspase-dependent apoptosis. However, our studies utilising pan-caspase inhibitor z-VAD-fmk do not completely abolish RV-induced apoptosis in RK13 cells. This suggested the involvement of other host cell signalling pathways, in particular those regulating cellular growth and survival. We investigated phosphatidylinositol 3-kinase (PI3K)-Akt and Ras-Raf-MEK-ERK signalling during RV infection and demonstrated that rubella infection causes an increase in the phosphorylation profiles of ERK, and of Akt and its downstream target GKS3 over time. Inhibition of PI3K-Akt signalling with pharmacological inhibitor LY294002 enhanced viral replication, reduced cell viability, and increased the speed and magnitude of RV-induced apoptosis. In contrast, complete cell cycle and host-cell shut-off by blockage of the Ras-Raf-MEK-ERK pathway with U0126 severely impaired RV replication and growth, and reduced RV-induced apoptosis. RV infection delays cell cycle progression, which was confirmed by the observation that RV gradually decreases the phosphorylation profile of transcription factor c-myc. These studies suggest that RV induction of apoptosis is accompanied by host-cell induction of PI3K-Akt and mitogen activated signalling. Although RV infection slows cell growth, it requires cells to be cycling and metabolising normally for efficient virus production using host cell components.

Plasticity of dendritic cell transcriptional responses to RNA viruses

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As obligate intracellular parasites, viruses utilise many cellular pathways and components during their replication cycle. In the dynamic environment that occurs during infection and the resulting immune response, host transcriptional changes reflect both virus-induced changes and host responses to infection.

To dissect the disparity between outcomes of different viral infections it is important to look at how viruses are controlled by the immune system. Dendritic cells (DCs) are the most potent antigen presenting cells in the immune system, specialised in their capacity to initiate adaptive immune responses. Their location at epithelial surfaces, mucosae and blood make immature DCs among the first cells exposed to viral particles. Their importance in shaping downstream adaptive immune respones, driving polarised T helper responses and cytotoxic CTLs, make DCs strategic targets for viruses in order to subvert the immune response.

Using DNA microarrays we have profiled transcriptional responses of DCs to Influenza infection and Rhinovirus exposure. This allows comparison of DC responses to two different single-stranded RNA viruses, one of which is enveloped, and DC responses to active viral replication and virus exposure. We have found that DCs are able to tailor transcriptional responses specific to the viral stimulus, and differentially respond to the two viruses, as well as activate a common interferon-mediated antiviral response.

Cellular responses to alphavirus infection

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Virus vectors based on the alphavirus Semliki forest virus (SFV) are showing promise as oncolytic agents, however the molecular events underlying tumour cell destruction remain to be determined. Cellular responses to infection with a virulent (L10) and an avirulent (A7(74)) strain of SFV were analysed at the molecular and cellular level. SH-SY5Y neuroblastoma cells were synchronised in G0/G1 by serum-deprivation prior to infection and were analysed by flow cytometry. SFV infection pushed cells into S phase, (23.4±8.5% (A7(74)) and 24.9±4.4% (L10) at 12 hours post infection (pi) compared to 3.2%±0.8% (uninfected)). Nuclear DNA fragmentation (apoptosis) was evident earlier, and in more cells, with the L10 strain (17.7±7.3% (L10) compared to 8.9±2.3% (A7(74)) and 0.9±0.4% (uninfected) at 12 h pi; and 40.5±4.3% (L10) compared to 31.1±4.1% (A7(74)) and 1.4±0.7% (uninfected) at 24 h pi). Using a cell cycle cDNA microarray, we observed up-regulation of GADD153, JunB and Cdk10 in response to infection with both strains of SFV. These results were confirmed by RT-PCR, and the Cdk10 transcript was further characterised as isoform P3.

Human cytomegalovirus UL141 encodes a novel inhibitor of NK cytotoxicity

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Human cytomegalovirus (HCMV) has played a major role in defining the activating and inhibitory receptors that regulate Natural Killer (NK) cells. Although the archetypal HCMV laboratory strains AD169 and Towne encode multiple NK evasion genes, NK killing is regularly observed against AD169/Towne-infected targets. In contrast, infection with low passage laboratory strains (e.g. Toledo, TB40) induces almost total resistance to *in vitro* NK lysis. We hypothesized that the large (13–15 kb) deletions within AD169/Towne may contain additional NK resistance genes, responsible for the enhanced protection. We now identify a gene from this region, UL141, as a NK evasion factor. Cells transfected with UL141 or infected with adenovirus vectors driving UL141 expression, acquired protection from NK lysis. Furthermore, the resistance of low passage HCMV strains to NK lysis was compromised following the loss of UL141 expression. This was shown in assays using polyclonal NK cells and NK clones against both allogeneic and autologous targets. UL141 is thus identified as a major HCMV immune evasion factor with potential clinical significance.

Co-ordination of virus resistance mechanisms by salicylic acid in plants

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The plant signal molecule salicylic acid (SA) can induce resistance to a wide range of pathogens, including viruses. SA can stimulate inhibition of all three main stages in virus infection: replication, cell-to-cell movement and long-distance movement. Induction of resistance by SA appears to depend, in part, on downstream signalling via perturbations in mitochondrial redox. However, evidence has recently emerged that SA may also stimulate a separate downstream pathway leading to the induction of a host gene encoding an RNA-directed RNA polymerase. This strongly suggests the existence of an additional SA-induced resistance mechanism based on RNA interference (RNAi). This broad spectrum of SA-induced anti-viral mechanisms may aid plants in dealing with diverse viruses with different strategies for replication, movement, or evasion of host defence.

Complete sequence analysis of the ovine herpesvirus 2 reveals a highly divergent but functional IL-10 homologue

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Ovine herpesvirus-2 (OvHV-2) is a γ-herpesvirus that causes malignant catarrhal fever in domestic cattle and deer. Our aim was to determine the complete sequence of OvHV-2 and identify genes contributing to the pathogenesis of MCF. OvHV-2 cannot be grown conventionally. Thus, overlapping cosmid clones containing most of the viral genome were generated from an OvHV-2-carrying lymphocyte line and sequenced. The sequence was then completed by splinkerette PCR. Sequence analysis, confirmed by cDNA cloning, revealed an IL-10-like gene at the left end of the unique portion of the genome. IL-10 homologues are encoded by many viruses. However, OvHV-2 vIL-10 is unusual since the gene contains 4 short introns (80–100bp), with similar intron/exon boundaries to the ovine IL-10 gene. Also, the overall amino-acid homology with mammalian and other viral IL-10s is low (~40% identity). In spite of this, OvHV-2 IL-10 exhibits anti-inflammatory and immuno-stimulatory activity typical of mammalian IL-10s. Thus, this gene may have been acquired recently and is evolving towards an intron-less sequence. This protein is likely to contribute to the pathogenesis of MCF. Further, comparing OvHV-2 IL-10 with mammalian and viral IL-10s has allowed identification of key residues involved in IL-10 activity.

Two distinct transport motifs in the adenovirus E3/10.4–14.5 proteins act in concert to down-modulate apoptosis receptors and the EGFR

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The adenovirus (Ad) early transcription unit E3 encodes immunosubversive functions. The E3 transmembrane proteins 10.4 and 14.5 form a complex (RID) that downregulates the EGFR and apoptosis receptors from the cell surface by diverting them to lysosomes for degradation. The latter protects infected cells from apoptosis. The mechanism by which 10.4–14.5 mediate rerouting remains elusive. We examined the role of putative YXXΦ and dileucine (LL) transport motifs within Ad2 10.4–14.5 for target protein modulation. By generating E3 transfectants expressing 10.4–14.5 proteins with alanine substitutions in these motifs, we show that 3 of the 5 motifs are essential for functional activity. While tyrosine 74 in 14.5 appears to be important for efficient 10.4 interaction, the ^{122}YXXΦ motif in 14.5 and the dileucine motif in position 87/88 of 10.4 constitute genuine transport motifs: disruption of either motif abolished binding to clathrin adaptor proteins AP-1 and AP-2 and dramatically altered RID cell surface appearance and intracellular location. FACS analysis and immunofluorescence data provide evidence that 14.5^{122}Y is essential for rapid RID endocytosis, whereas 10.4LL acts downstream and protects RID from extensive degradation by rerouting it into a recycling pathway. Infection of primary cells with Ads carrying the relevant point mutations confirmed the crucial role of these transport motifs for down-regulation of target receptors (1). Thus, two distinct transport motifs present in two proteins synergize for efficient target removal and immune evasion.

Reference: (1) Hilgendorf *et al.*, *J Biol Chem* (in press).

Immunomodulation by the African swine fever virus A238L protein

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African swine fever virus (ASFV) is a large double stranded DNA virus which, while asymptomatic in its natural hosts, the warthog and bushpig, causes an acute haemorrhagic disease in domestic swine. Previous work has identified a viral protein, A238L, which acts as a functional homologue of IκB, the cellular inhibitor of

NF-κB. We established previously that A238L was also capable of inhibiting the phosphatase activity of calcineurin, inhibiting activation of the NFAT pathway. A238L is synthesized during infection as two molecular weight forms of 28 kDa and 32 kDa. Both forms of the protein are synthesized throughout infection and the 32 kDa form accumulates in the nucleus at late times post-infection. The 32 kDa form of the protein has been shown to co-precipitate with the NF-κB p65 subunit. Experiments studying the localization of NF-κB during ASFV infection showed that A238L expression does not inhibit nuclear translocation of NF-κB suggesting that A238L functions by inhibiting NF-κB activity within the nucleus. We have also shown a role for A238L in inhibiting cell proliferation in culture. Further work aims to characterize the mechanisms of these effects.

Type-1 interferon and the pathogenesis of Semliki Forest virus (SFV)

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Type-1 interferons, (IFNα/β), are potent mediators of anti-viral innate immune responses. SFV is a positive-stranded RNA virus of the *Togaviridae*. In immunocompetent adult mice, SFV4 is virulent whereas, SFV A7(74) is avirulent; both strains induced strong interferon responses in peripheral tissues and on replication in the brain. IFNα/β act via a cell-surface receptor (IFNα/β-R) to induce a number of anti-viral defence proteins; these include the dsRNA-dependent protein kinase (PKR), 2–5A synthetase/RNase-L and the Mx proteins. The protective role of this system against SFV was studied by comparing the course of infection in IFNα/β-R$^{o/o}$, PKR$^{o/o}$, RNase-L$^{o/o}$, IFNα/β-R$^{o/o}$/MxA$^{+/+}$ and control (wt) mice. A functional type-I interferon system was required to protect adult mice from avirulent A7(74) infection. However, this protection required neither PKR nor RNase-L and MxA alone was insufficient. In the absence of a type I interferon response, SFV was widely disseminated in peripheral tissues. In the CNS, virus spread in meningeal, ependymal and glial cells but restricted neuronal replication of the A7(74) strain was unaffected.

Signal-dependent degradation of transcriptional regulators in adenovirus infected cells

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The transcription factors p53 and NF-κB are important determinants of a finely balanced control system that determines if cells will live or die. Various stress and genotoxic insults activate both p53 and NF-κB but these transcription factors have antagonistic effects, with p53 inducing a set of proapoptotic genes and NF-κB inducing a set of anti-apoptotic genes. The strength of signal through these separate pathways determines the fate of the cell. When human cells are infected with adenovirus, the E1A genes drive the cell into S-phase and in doing so activates p53. However the virus blocks apoptosis by inhibiting and ultimately degrading p53. Here we describe the mechanism by which the viral proteins E1B 55K and E4 orf6 target p53 for degradation. Surprisingly p53 is degraded by a mechanism that is proteasome dependent, but ubiquitin-independent. In addition to inducing p53, adenovirus E1A also activates NF-κB. Here we determine the pathway of NF-κB activation and demonstrate that the NF-κB inhibitor IκBa is inactivated by polyubiquitination, but does not appear to undergo proteasomal degradation. Thus the hypothesis we have developed is that adenovirus blocks ubiquitin dependent proteolysis, but induces degradation of key proteins by directly targeting them to the proteasome.

Functional analysis of the novel influenza virus protein PB1-F2

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Abstract not received

Perturbation of cell surface receptors by human cytomegalovirus – a means to ensure cellular responses to virus-specific signals

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Infection with human cytomegalovirus (HCMV) results in complex interactions between viral and cellular factors effecting disruption of a number of cellular functions. Specifically, HCMV infection is known to target control of cell cycle, cellular transcription and immunoregulation; presumably, to optimise the cellular environment for viral persistence and productive infection. We will describe how HCMV infection also inhibits external signalling to the cell by disrupting the expression of at least three cellular receptors; the receptors for tumour necrosis factor (TNFRI), epidermal growth factor (EGFR) and the CC-chemokine receptor 5 (CCR5).

Infection with HCMV results in no change in the steady state levels of TNFR1 expression but induces the relocalisation of TNFRI from the cell surface to the Golgi architecture. Intriguingly, whilst HCMV infection also results in disruption of EGFR expression, in contrast to TNFRI, this correlates with a virally-induced decrease in steady state levels of EGFR.

Furthermore, we will describe how HCMV can also decrease expression of CCR5 on the infected cell but upregulates CCR5 expression on uninfected by-stander cells.

We suggest that a primary goal of HCMV is to 'isolate' the infected cell from host-mediated signals so that the cell responds solely to an array of virus-specific signals which optimise the cell for virus production.

Measles virus: immunosuppression by interference with T cell signalling and activation

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Surface contact with the MV glycoproteins is known to abolishe mitogen-driven expansion of T cells and this is characterised by deregulations of S phase entry control proteins. On a molecular level, TCR dependent raft recruitment and activation of phosphoinositol-3 kinase (PI3K) is abolished in T cells after MV binding as is the recruitment of the guanosine exchange factor Vav, GTP loading of Rho family proteins and reorganisation of the actin cytoskeleton. CD3/CD28-induced Ca flux and overall tyrosine phosphorylation are inhibited 24 hours after MV/T cell interaction coinciding with the onset of measurable inhibition of T cell proliferation. Also 24 hrs after MV treatment, we observed production of alternatively spliced mRNAs for the regulatory subunit of the PI3K and the phosphoinositol-phosphatase SHIP, and this likely alters the adaptor function of these proteins in T cell signaling. Using a reporter system where expression of the reporter (luciferase) depends on TPA/Ionomycin stimulation, we found that both the vaccine strain and wild type MV prevented splicing in primary T cells in a dose dependent manner. Initial viral glycoprotein contact with the cell membrane thus profoundly affects TCR signaling and inducible splicing of cellular mRNAs and this might be crucial in the understanding of MV-induced immunosuppression.

Paramyxoviruses, interferons and virus : host cell interactions

R.E. Randall

University of St Andrews

Many paramyxoviruses circumvent the interferon (IFN) response by blocking both IFN signaling and IFN production. In the case of Simian Virus 5 (SV5), the virus blocks IFN signaling by targeting STAT1 for proteasome-mediated degradation. To further define the molecular interactions involved in this process we have recently developed an *in vitro* ubiquitination and degradation assay. Central to this process, is the V protein of SV5, as well as the cellular proteins STAT2, the p127 subunit of the DNA damage binding protein (DDB1) and Cullin 4a. These proteins form part a multiprotein complex which is involved in the ubiquitination of STAT1. As well as blocking IFN signaling, the V protein of SV5 also inhibits IFN production. The functional domains on V involved in blocking IFN production overlap but are different from those required to target STAT1 for degradation. With regards inhibition of IFN production, the transcription factors NF-_B and IRF3 are not activated in cells infected with wild type SV5, but are activated in cells infected with a mutant virus (SV5V∏C) that makes a truncated version of V and induces the production of large amounts of IFN by infected cells. Furthermore, the V protein of SV5 can act *in trans* to block dsRNA and SV5V∏C induction of IFN. On a practical side, we have used the information generated from these studies to engineer cell-lines which are non-responsive to IFN. The practical applications of using such cells in vaccine development and manufacture, virus diagnostics and isolation of newly emerging viruses, and studies on host cell tropism and pathogenesis will briefly be discussed.

Transport of African Swine Fever virus from virus assembly sites to the cell surface is dependent on microtubules and conventional kinesin

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African Swine Fever virus (ASFV) is a large DNA virus that assembles in perinuclear viral factories located close to the microtubule organizing center. Following assembly virus particles move through the cytoplasm to the cell surface by an unknown mechanism prior to budding at the plasma membrane. In this study, we have investigated the mechanism by which ASFV reach the cell surface from their perinuclear site of assembly. Immunofluorescence microscopy revealed that at 16 hours post infection virus particles were aligned along cytoplasmic microtubules and that their movement to the cell periphery was inhibited when microtubules were depolymerised by nocodazole. In addition we found that ASFV infection increased both the stability of microtubules against depolymerization by nocodazole and their hyperacetylation. Immunofluorescence microscopy showed that conventional kinesin was recruited to both virus factories and virus particles in the cytoplasm. Consistent with a role for conventional kinesin during ASFV egress to the cell periphery, we found that overexpression of the cargo-binding domain of the kinesin light chain severely inhibited movement of particles to the plasma membrane. Based on our observations we propose that ASFV is recognised as cargo by conventional kinesin and uses this plus-end microtubular motor to move from perinuclear virus factories to the cell surface.

Herpes simplex virus type 1 capsid protein VP26 interacts with dynein light chain RP3 and plays a role in retrograde cellular transport

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Retrograde transport of herpes simplex virus type 1 (HSV-1) in axons is thought to involve the microtubule-associated motor cytoplasmic dynein, but the viral and cellular proteins involved are not known.

A library of HSV-1 capsid and tegument structural genes was constructed and tested for interaction with dynein subunits in a yeast two-hybrid system. A strong interaction was demonstrated between the HSV-1 outer capsid protein VP26 (UL35), as well as the tegument protein VP11/12 (UL46), with the homologous dynein 14 kDa light chains RP3 and Tctex1. VP26 is ideally located to bind dynein, since most of the tegument is believed to dissociate from the capsid following cell entry.

In vitro pull-down assays confirmed binding of VP26 to RP3 and Tctex1. Recombinant HSV-1 capsids +/– VP26 were used in similar pull-down assays. Only VP26+ capsids bound to RP3. Recombinant HSV-1 capsids were microinjected into Hep2 cells and incubated at 37°C. After 2 or 4 h VP26+ capsids had moved closer to the cell nucleus, while VP26– capsids remained in a random distribution. Thus VP26 mediates binding of incoming capsids to the retrograde motor dynein during cellular infection, through interactions with light chain RP3.

Baculovirus egress requires F-actin and cortical microtubules

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The *Baculoviridae* are a family of large DNA viruses that infect mainly lepidopteran hosts. Once replication of the virus in the host cell's nucleus is complete, newly formed capsids bud through the nuclear membrane acquiring a lipid envelope. In the cytoplasm this envelope is lost. This probably occurs in order to facilitate transport of the capsids to the plasma membrane by interactions between capsid proteins and components of the host cytoskeleton. Early studies on baculovirus trafficking demonstrated the involvement of F-actin in the entry of capsids, however, little is known about baculovirus capsid egress. In the present study, we have used confocal and electron microscopy in combination with yeast two-hybrid, immunoprecipitations and spin-down assays to demonstrate the involvement of microtubules and F-actin in egress of baculovirus capsids. These studies have indicated that protein-protein interactions between the capsid proteins VP39, VP80 and VP87 (ORF 1629), with α-tubulin or β-actin.

Genome-wide predicted RNA Structure (GPRS): implications for RNA virus evolution and persistence

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Using new bioinformatic analysis methods we present evidence for large scale structured ordering of many positive-strand animal and plant RNA virus genomes, distinct from discrete secondary and higher-order RNA structures often involved in virus replication. The presence of genome-wide predicted RNA structure (GPRS) varies remarkably between virus genera and families, for example between genera within *Flaviviridae* and *Picornaviridae*, and within many plant virus families. The similarity in the replication strategies between genera in these families rules out roles for GPRS in fundamentally conserved aspects of the virus life cycle. However, the existence of GPRS correlated strongly with their ability to persist in their natural hosts, raising the intriguing possibility of a role for GPRS in the modulation of the activity of intracellular defence mechanisms, perhaps through an interaction with the dsRNA recognition pathways. Irrespective of function, the observed evolutionary conservation of GPRS in many viruses imposes a considerable constraint on genome plasticity and the consequent narrowing of sequence space in which neutral drift can occur. Constriction of sequence space potentially reconciles contrasting aspects of RNA virus evolution; rapid sequence change over short periods but evidence for extreme conservatism evident from documented examples of RNA virus/host co-evolution.

Sequential modification of translation initiation factor eIF4GI by two different foot-and-mouth disease virus proteases within infected cells

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Infection of cells by foot-and-mouth disease virus (FMDV) inhibits cellular cap-dependent protein synthesis. This results from the cleavage of translation initiation factor eIF4G within the cap-binding complex eIF4F. This heterotrimer also contains eIF4E (which binds to the 5' terminal cap-structure of mRNAs) and eIF4A (which has RNA helicase activity). The complex acts as a bridge between the mRNA and the 40S ribosomal subunit. Two FMDV proteins, the Leader and 3C proteases induce cleavage of eIF4G at distinct sites within BHK cells. Within FMDV-infected BHK cells, sequential cleavage of eIF4GI by the L and 3C proteases occurs. The FMDV 3C cleavage site within eIF4GI was localised to a small region (about 40 residues) of the protein, between the sites cleaved by poliovirus 2A protease and the HIV-2 protease. Human eIF4GI is resistant to the action of FMDV 3C. On the basis of amino acid sequence alignments, it was predicted and then verified that substitution of a single amino acid residue within this region of human eIF4GIa conferred sensitivity to cleavage by FMDV 3C. The full-length eIF4GI and both forms of the C-terminal cleavage product must be capable of supporting the activity of the FMDV IRES in directing translation initiation.

Molecular pathogenesis of Coxsackievirus infections

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Coxsackieviruses, members of the Enterovirus genus of Picornaviridae, cause a wide spectrum of diseases including respiratory infections, rashes, myocarditis and meningitis. Since most enteroviruses have similar clinical manifestations in humans, and the same serotype can cause diverse symptoms, it is difficult to investigate enterovirus pathogenesis. However, insights into pathogenicity were obtained using recombinant viruses constructed between coxsackievirus A9 (CAV9) and coxsackievirus B3 (CBV3).

The five recombinants constructed contained different combinations of the CAV9 and CBV 5'NCR, capsid and non-structural regions. They were examined in a mouse model to map genetic determinants underlying the observed differences in pathogenicity and tissue tropism of CAV9 and CBV3. Using plaque titration we found that the CBV3 structural genes were required for liver infection, whereas the 5'NCR of CBV3 enhanced replication in the pancreas. A chimera, containing the CBV3 capsid and the rest of the genome from CAV9, and also parental CBV3, caused extensive viremia in adult mice, and were the only viruses in the panel able to infect the CNS. In addition, the presence of CBV3 capsid proteins induced high levels of neutralising antibodies. The results indicate complex enterovirus-host interactions, with different genomic regions influencing pathogenesis, although the capsid region plays a major role.

A chimaeric bovine enteric calicivirus: implications for the diagnosis and immunological control of noroviruses

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The Norovirus genus of the *Caliciviridae* encompasses viruses that cause high profile outbreaks of gastroenteritis in man and have been associated with diarrhoea in cattle. The 2 bovine noroviruses, Bo/Newbury2/76/UK and Bo/Jena/80/DE, represent 2 distinct genetic clusters in the newly described genogroup III. In the present study, a heteroduplex mobility assay identified Jena-like polymerase sequences for the first time in the UK but one of these, Bo/Thirsk10/00/UK, was a chimaeric virus. Molecular analysis of a 2818-nucleotide sequence generated from the 3' end of the genome showed that Bo/Thirsk10/00/UK had a Jena-like polymerase gene but Newbury2-like capsid and ORF3 genes by comparison of their genome organization, nucleotide and amino acid identities plus phylogenetic analyses. The recombination point for Bo/Thirsk10/00/UK was identified at the ORF1–2 (polymerase-capsid) overlap. The present study is one of few studies to clearly demonstrate the existence of chimaeric genomes in the Norovirus genus and the first to identify a chimaeric genome in genogroup III. This strongly supports the notion that recombination is part of the natural evolution of the Norovirus genus of the *Caliciviridae*. These findings are highly relevant to the diagnosis and immunological control of norovirus diarrhoea outbreaks.

An approach to determining cellular genes important in host restriction of avian influenza viruses using a radiation hybrid system

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Influenza A viruses have a reservoir of infection in wild waterfowl. Most avian influenza viruses undergo very limited or abortive replication in mammalian cell culture. Accordingly, only

a few subtypes are established pathogens in mammalian species and adaptation of the virus is required for efficient replication and transmission to occur. Many aspects of the cellular basis for this host range restriction are still unclear.

We have developed a system to investigate host cell specific genes involved in influenza A virus replication. A panel of radiation hybrid cell lines has been assayed for competent replication of an avian influenza virus, A/Turkey/England/50-92/91 (H5N1), which undergoes abortive replication in the parental Wg3H hamster cells. The panel was generated by random insertion of irradiated chicken embryo fibroblast genome into the Wg3H hamster cell line. Five hybrid cell lines have been identified as permissive to multi-cycle propagation of this virus.

Experimental data is being gathered to identify at what stage in the viral life cycle the barrier to virus replication is overcome in these positive hybrids. Micro-array technology is being used to identify candidate genes that might contribute to the ability of the hybrids to support productive replication of this avian influenza A virus.

Attenuation of bunyavirus replication by genome rearrangement

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Bunyamwera virus (BUN) is the prototype of the genus *Orthobunyavirus* and the family *Bunyaviridae*. BUN has a tri-partite genome of negative sense RNA, composed of small (S), medium (M), and large (L) segments. Partially conserved and complimentary untranslated regions (UTRs) flank the coding region of each of the genome segments. The UTRs direct replication, transcription, encapsidation, and packaging of the viral RNA. Using reverse genetics we have recovered a recombinant virus in which the L segment open reading frame is flanked by M segment UTRs (called BUN MLM). BUN MLM shows growth attenuation in cultured mammalian and mosquito cells; has a small plaque morphology; expresses reduced levels of L mRNA and L (RNA polymerase) protein; synthesizes less antigenomic and genomic RNA; and has an increased particle to pfu ratio relative to wt BUN. The implications of these findings will be discussed.

Plant viruses

Host factors mediating susceptibility to potyvirus infection

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In common with all plant viruses, members of the genus Potyvirus (family *Potyviridae*) comprise a collection of modular functional units that mediate the generic processes of genome replication and expression, encapsidation, cell-to-cell movement, and suppression of host defences. These units need not be represented by single gene products and in many cases the functional roles of several products overlap. Relatively little is known about how these units are integrated into and/or exploit host functions to achieve a productive infection. We have studied the properties of a small genome-linked viral protein (VPg) that is covalently linked to the 5' end of the genomic RNA. This protein has previously been implicated in RNA replication, translation, and virus movement. We have shown that this protein can interact *in vivo* with several host-encoded proteins. These interactions provide further evidence for the involvement of VPg in viral gene expression and virus movement, and illustrate how the disruption of such interactions can lead to virus resistance.

Viral hepatitis

Overview of liver architecture and pathology

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The liver plays many pivotal roles in intermediary metabolism and in the clearance of toxins. Various models have been described for the basic functional unit in the liver including the classical lobule, acinus, primary lobule and hepatic microcirculatory subunit. The parenchyma comprises epithelial cells (hepatocytes and cholangiocytes), endothelial cells (vascular and fenestrated sinusoidal types), macrophages (Kupffer cells), (myo)fibroblast like cells (hepatic stellate cells) and liver associated lymphocytes. There are crucial functional interactions between each of these cells types and the surrounding matrix.

The liver is susceptible to injury by exogenous toxins (e.g. alcohol), infections (most notably HBV and HCV), autoimmunity and ischaemia. Injury may also occur through inborn or acquired metabolic disorders and disturbance to bile flow. Despite the large number of aetiologies, there are a limited number of ways that the liver responds. In broad terms we can distinguish between *acute injury* (e.g. paracetamol toxicity) where there is a 'single hit' necrosis (+ apoptosis) of hepatocytes followed by transient inflammatory and regenerative responses leading to complete resolution and *chronic injury* (e.g. HCV infection) where liver cell loss occurs over a prolonged period with persistence of inflammation. This leads to activation of the hepatic stellate cells (and other mesenchymal cells) with the production of excess extracellular matrix and the development of fibrosis. This is accompanied by a regenerative response by hepatocytes and formation of nodules (cirrhosis); clinically this leads to liver failure and portal hypertension. Although it was previously thought that cirrhosis was irreversible, there is now evidence to suggest that if the injurious agent is removed there can be substantial remodelling of the liver architecture.

Acute hepatitis (A and E): recent advances in molecular biology and disease pathogenesis

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Hepatitis A (HAV) and E (HEV) viruses are the primary agents responsible for acute viral hepatitis. Both viruses have similar modes of transmission and endemic regions yet show distinct epidemiological features.

The HAV is a picornavirus with a 7.5 kb positive-strand RNA genome that is translated into a polyprotein and subsequently processed into 11 different protein products. These include the VP1-4 structural components of the capsid, as well as the nonstructural proteins involved in genome replication and polyprotein processing. A replicon system has recently been established. Other recent developments, including characterization of a cellular receptor, imaging of viral replication and effects on the tubular-vesicular network of infected cells suggest that HEV is a unique picornavirus. A cryo EM structure for HAV may provide clues to particle stability and slow uncoating. An attenuated cell culture adapted vaccine is available.

The HEV is an unclassified virus with a 7.2 kb positive-strand RNA genome with three open reading frames (ORFs). The ORF1 translates into a nonstructural polyprotein with distinct motifs but little biochemical characterization or processing information. The viral capsid protein encoded by ORF2 also shows interesting nonstructural functions. The ORF3 protein modulates the cellular environment through signaling pathways. The replication of genomic RNA in animal and *in vitro* systems as well as the development of a subgenomic replicon has been new developments. An experimental vaccine for HEV has recently undergone human trials.

I will cover some basic aspects of HAV and HEV biology, and emphasize new developments in the field, especially those related to HEV.

Chronic hepatitis – comparison of the clinical aspects and course of infection HBV/HCV

G. Alexander

University of Cambridge

Abstract not received

Hepatitis B virus replication and infection – new insights from *in vitro* and *in vivo* studies

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Chronic hepatitis B virus (HBV) infection continues to be a major public health problem. The limited efficacy of current therapies is further put at risk by the emergence of resistant virus variants. Improving this situation requires a much more thorough understanding of the basic biology of the virus but is faced with two major bottlenecks: as yet there is neither a feasible cell culture infection system or small animal model to propagate HBV *in vivo*, nor is there an vitro replication system to study the reverse transcriptase of HBV by biochemical means. Fortunately, a naturally occurring close relative of HBV, duck hepatitis B virus (DHBV), provides us with a much less restricted model system. Recent methods to recombinantly express DHBV reverse transcriptase have allowed its *in vitro* reconstitution with purified chaperones into enzymatically active replication complexes. Primary duck hepatocytes, and ducks, on the other hand, offer a practical means for *in vivo* studies to shed more light on the poorly understood early steps of infection. Applying this system to the elucidation of the narrow host range of hepadnaviruses we have recently obtained rather unexpected results showing that the current view of the molecular host range determinants requires major, if not fundamental, revisions.

Immunopathogenesis of HBV infection

A. Bertoletti

University College London

Abstract not received

Vaccinology of HBV

K. Murray

University of Edinburgh

Abstract not received

Replication of hepatitis delta virus

J. Taylor, J. Chang, S. Gudima, X. Nie & C. Tarn

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Hepatitis delta virus (HDV) is a sub-viral agent that uses hepatitis B virus as helper. Replication of the 1,700-nucleotide single-stranded circular HDV RNA genome is described via a double rolling-circle model, with RNA-directed RNA synthesis achieved by redirection of host RNA polymerase II. Replication produces an exact complement of the genome, the antigenome, and a less than full-length polyadenylated species, which acts as mRNA for translation of an essential protein, the 195 amino acid small delta antigen. This presentation describes results of five recent studies. (i) Small interfering RNAs (siRNA) to the mRNA can block HDV replication. (ii) HDV RNAs are resistant to the endonuclease *dicer*, both *in vivo* and *in vitro*. (iii) Examination of the processing of nascent antigenomic RNA transcripts provided results that demand modifications to the rolling-circle model. (iv) Inter- and intra-molecular competition assays were used to reveal sequence and structural requirements of HDV RNA species that can initiate replication. (v) Using conditional expression of the essential small delta protein, the replication of the HDV genome was found to be directly cytopathic. And yet, with restricted amounts of delta protein, replication was maintained for greater than 6 months and allowed studies of HDV genome evolution.

HCV – historical perspective and vaccine development

M. Houghton

Chiron Corporation, Emeryville, California, USA

Abstract not received

HCV – molecular biology of sp RNA replication

C. Rice

Rockefeller University, New York, USA

Abstract not received

HCV – assembly, particle formation

A. Patel

University of Glasgow

The structural proteins of hepatitis C virus (HCV) – the capsid or core protein and the envelope glycoproteins E1 and E2 – constitute the components of the virion. The virus particle likely consists of a host-derived membrane containing E1 and E2 glycoproteins that envelope the nucleocapsid which is assembled by polymerisation of core and genomic RNA. Due to lack of a permissive culture system for HCV, the biogenesis of viral structural proteins has been studied exclusively in heterologous expression systems in which the glycoproteins are targeted to the ER where the virus particle is thought to acquire its envelope. Similar observations have been reported in human hepatoma cells harbouring genome length viral replicons, although particle assembly is not detected. In contrast, expression of core, E1 and E2 in insect cells leads to assembly of virus-like particles that are at least partially enveloped with bilayer membranes and viral glycoprotein spikes protruding from the surface. HCV core, in addition to its role as a nucleocapsid, also interacts with numerous host cell factors, some of which may be involved in virus particle assembly. This presentation will focus on our current understanding of the interplay between the viral proteins (and host cell factors) which are likely to be crucial for particle assembly.

HCV therapeutic approaches

Tony Carroll

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Hepatitis C virus (HCV) is a major cause of chronic liver disease worldwide, with approximately 3% of the world's population being infected. In the United States it is the most common indication for orthotopic liver transplantation in adults. Therapies for the treatment of hepatitis C have improved over the last decade with the current combination of pegylated interferon and ribavirin achieving a sustained response in more than 50% of patients in clinical trials. However this therapy has significant side effects and there is a need for a more effective and better tolerated anti-HCV agent(s). We will review HCV gene products that may be potential targets for small molecule anti-viral activity. In particular we will concentrate on efforts at GSK to develop small molecule anti-HCV agents against the major enzymes encoded by the virus. Specifically we will discuss attempts to find inhibitors of the NS3 protease, the NS3 helicase and the NS5B RNA polymerase. Furthermore, since there is no reliable cell culture system for HCV we will discuss the role of the recently developed sub-genomic replicon system in the development of anti-viral compounds and the use of other surrogate systems used in this process.

Posters

Cells & Cell Surfaces Group

CCS 01 Identification and functional analysis of three protein disulphide oxidoreductases of *N. meningitidis*

S. Sinha, P. Langford & J.S. Kroll

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Neisseria meningitidis is an invasive pathogen capable of causing life-threatening meningitis and septicemia, and is responsible for many deaths worldwide.

DsbA, a thiol-disulphide oxidoreductase, was identified during a search for meningococcal-specific virulence determinants. DsbA ensures the correct folding of many exported bacterial proteins by forming intramolecular disulphide bonds. Through the folding of virulence factors, DsbA has been implicated in the virulence of many bacterial pathogens.

Inspection of the *N. meningitidis* MC58 genome sequence revealed three putative *dsbA* genes (NMB0278, NMB0294 and NMB0407). This is unprecedented as even bacterial species closely related to *N. meningitidis* possess only a single *dsbA* gene on the chromosome.

All three cloned meningococcal *dsbA* genes successfully complemented a *dsbA*-deficient *E. coli* strain, restoring phenotypes for alkaline phosphatase activity, motility and DTT sensitivity. In addition, in a model system, all three meningococcal DsbA proteins restored the correct folding of MalF, a periplasmic protein.

Knockout mutants of individual and multiple *dsbA* genes in *N. meningitidis* have been constructed. These strains, with wild-type, will be used to study the role of the different DsbAs in meningococcal biology and virulence.

CCS 02 Identification and characterization of regulators of region 1 promoter of *Escherichia coli* K5 capsule gene cluster

H. Askar & I.S. Roberts

School of Biological Sciences Manchester University

E. coli, can express a polysaccharide that is an important virulence factor in extraintestinal infections. Based on genetic criteria, there are 4 groups of *E. coli* capsules; 1, 2, 3, and 4 with most pathogens expressing a group 2 capsule. The *E.* coli K5 capsule is the paradigm for group 2 capsules. K5 capsule gene cluster is composed of three regions; 1,2, and 3 Expression of K5 capsule gene cluster is controlled by two convergent promoters one (P1) is 5' to region 1 and the other (P3) is 5' to region 3. To study P1 we constructed a single-copy chromosomal P1-*lac*Z fusion strain (HA1) and found that this promoter is temperature regulated. To determine the effect of some regulators as BipA and H-NS, we designed mutants and we concluded that H-NS and BipA are necessary for maximal expression of region 1 at 37°C. At 20°C H-NS mutation enhanced transcription from P1. Transposon mutagenesis was also applied to HA1. 11 mutants with decreased b-Galactosidase activity were isolated and characterized. Out of these there were 2 *BipA* mutants. A plasmid library was then constructed in an attempt to find out some genes that can suppress the *BipA* mutation. So far two genes were identified and their further characterization is going on.

CCS 03 Seven-strain *S. aureus* whole genome microarray: design, construction and validation

Jodi Lindsay[1], Gemma Marsden[1], Adam Witney[1], Matt Holden[2], Jason Hinds[1], Sally Husain[1], Richard Stabler[1] & Philip Butcher[1]

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The Bacterial Microarray Group at St George's Hospital Medical School (BμG@S) is funded to design, build and print 12 whole genome bacterial microarrays. BμG@S collaborate with research groups, providing microarray slides and the expertise and equipment to use them (http://bugs.sghms.ac.uk). We have now designed and built a multi-strain *S. aureus* PCR-product microarray based on the ORFs of EMRSA-16 strain 252, supplemented with the novel ORFs found in the other 6 sequencing projects: MSSA strain 476, N315, Mu50, MW2, COL and 8325. These arrays are being used by ourselves and collaborators for DNA-DNA hybridisation studies, allowing rapid identification of the gene complement carried by particular isolates. They are also being used for gene expression profiling, allowing characterisation of regulatory mutants and the impact of various environmental conditions.

CCS 04 Interaction of LsaA, a *Lawsonia intracellularis* adherence-associated protein of the TlyA family, with intestinal epithelium

G.E.M. McAllister[1,2], M.P. Alberdi[1,2], E. Paxton[1,2] & **D.G.E. Smith**[1]

[1]Functional Genomics, Moredun Research Institute; [2]ZAP Research Laboratories, University of Edinburgh

Lawsonia intracellularis (*L-ic*) is an obligately intracellular bacterium with a specific tropism for immature intestinal epithelial cells. In contrast to other enteroinvasive bacteria (e.g. *Shigella*, *Listeria*, *Salmonella*) *L-ic* infection does not lead to inflammatory/apoptotic lesions but rather produces hyperplasia in infected crypts. Little is known of the molecular basis to *L-ic* pathogenesis however we have identified LsaA, an orthologue of TlyA, a family of proteins that has been reported in a wide range of bacterial species including *Campylobacter jejuni* and *Helicobacter pylori*. Despite the recognition of this protein family, function(s) has yet to be fully defined. LsaA is a surface protein which has been correlated with with adherence/invasion of epithelium. Immunoprecipitation, affinity purification and yeast 2-hybrid approached have been applied to characterise potential ligands for LsaA. Transfection of cells with plasmids encoding LsaA-GFP and TlyA-GFP have demonstrated localisation to subcellular compartments. Function is being assessed both by complementation of *tlyA* deletions in *H. pylori* and by challenge of epithelial cells with *lsaA* and *tlyA*. These studies are leading to improved understanding of LsaA/TlyA and its possible role during infection.

CCS 05 Role of H7 flagellin in interactions of *E. coli* O157 : H7 with ruminant intestinal epithelium

N. Paton[1,2], A. Mahajan[1,2], R.M. Laragione[3], M.J. Woodward[3], C.G. Currie[1], D.E.E. Hoey[1,2], D.L. Gally[2] & **D.G.E. Smith**[1]

[1]Functional Genomics, Moredun Research Institute; [2]ZAP Research Laboratories, University of Edinburgh; [3]Food and Environmental Safety, Veterinary Laboratories Agency, Addlestone

Enterohaemorrhagic *Escherichia coli* (EHEC), particularly those of serotype O157:H7, remain an important cause of foodborne zoonosis. Infection in Man can be severe or even fatal whereas in reservoir hosts such as cattle, infection is characteristicaly asymptomatic. Recent studies have identified flagella as important determinants of initial interactions between pathogenic *E. coli* and human epithelium including roles for these in cellular attachment. Significantly, flagellin represents a PAMP (pathogen-associated molecular pattern) which interacts with the PRR (pattern recogniton receptor) TLR5. Engagement of TLR5 by H7 (or other) flagellin results in activation of the transcription factor NF-κB with consequent expression of IL-8 as well as other genes. Through a combination of *in vitro* and *in vivo* challenges, we have examined the role of flagellin of *E. coli* O157:H7 in bacterial interaction with and induction of chemokine expression by epithelium – H7 showed roles in both.

Posters

Clinical Microbiology Group

CM 01 Bactericidal activity of honey on *Pseudomonas aeruginosa*

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The emergence of antibiotic resistant bacteria, especially multi-resistant *Pseudomonas aeruginosa,* requires the use of innovative antimicrobial strategies. For wound management honey is a possible topical agent with broad spectrum of activity, but before acceptance into conventional medicine its mode of action must be investigated *in vitro*. This study aims to explore the bactericidal action of a selected New Zealand honey (manuka) on *Pseudomonas aeruginosa*. Using ATTC 27853 *Pseud. aeruginosa* Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were determined and the effect of serum protein was investigated. Time-kill studies were performed with 5 clinical isolates of *Pseud. aeruginosa*, and *Pseud. aeruginosa* ATTC 27853 in 20% manuka honey in nutrient broth. The MIC's increased in the presence of serum proteins, but the MIC/MBC ratio both in the presence and absence of serum proteins was still consistent with bactericidal activity. All cultures showed at least 5 decimal reductions within 24 hours, with variable rates of death for different strains, and this supports a bactericidal mode of action. These results support further clinical evaluation of the use of honey as an alternative antimicrobial agent for infected wounds.

CM 02 Enhanced survival of MRSA and *Listeria monocytogenes* in association with *Acanthamoeba polyphaga*

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Since the origins of life bacterivorous protozoa and bacteria have been in co-existence, which has unequivocally led to the evolution of many different co-interactions. Most bacteria are ingested and digested, but many escape ingestion for various reasons. Others are ingested but evade digestion, and a few, notoriously *Legionella pneumophila*, have the capacity of multiplying within the protozoan host. The aims of this study were to elucidate the interactions of multi-drug resistant *Staphylococcus aureus* (MRSA), *Listeria monocytogenes* sv4b, *Enterococcus faecalis* and *Escherichia coli* with the amoeba, *Acanthamoeba polyphaga*. It was discovered under our test conditions, that both the *E. coli* and *E. faecalis* strain used were ingested and digested by *A. polyphaga*, but in contrast, *L. monocytogenes* sv4b, had the capacity to multiply intracellularly within *A. polyphaga*. We also report, for the first time, that all 6 MRSA isolates tested, survived and replicated in association with *A. polyphaga*. These findings have profound implications for the hospital environment, where *Acanthamoeba* sp., are also commonly isolated. In conclusion, this study illustrates the significance of protozoa as vehicles augmenting the survival of MRSA and *L. monocytogenes* in the environment.

CM 03 Worm-derived peptide prevents the growth of *Mycobacterium bovis* BCG and *M. tuberculosis*

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ASABF (*Ascaris suum* antibacterial factor) has previously been shown to be bactericidal for both Gram positive and Gram negative bacteria, and also posses anti-yeast activity. We decided to investigate the effect of ASABF on the growth of *Mycobacterium bovis* BCG (pasteur) and *M. tuberculosis*. Mycobacteria were cultured for 0–72 hours with recombinant ASABF at concentrations of 0–100 µg/ml. Mycobacterial growth was monitored at various time points (0, 24, 48 and 72 hours) by either spectrophotometry or by plating out on modified 7H11 agar plates. Results show that ASABF at 1 µg/ml prevented the growth of *M. bovis* BCGp, while a higher concentration of ASABF (100 µg/ml) was required to prevent the growth of *M. tuberculosis*. These data show that an antibacterial peptide produced by a parasitic helminth can modulate the growth of mycobacteria, and suggests how worms may directly affect mycobacteria within the host. Furthermore, this and other worm-derived peptides may have therapeutic potential in the treatment of tuberculosis.

CM 04 Serotype and clonal analysis of invasive pneumococci in Scotland

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Until recently, only limited molecular epidemiological information has been available for invasive pneumococcal disease (IPD). New pneumococcal conjugate vaccines have been developed, one of which has gained licensure in both Europe and the US, and the need for improved epidemiological data is now evident.

Multi-locus sequence typing (MLST) is an unambiguous nucleotide sequence-based typing method for characterising bacteria using the sequences of internal fragments of seven house-keeping genes. MLST provides molecular typing data that is highly discriminatory and electronically portable between laboratories. Since January 2003, MLST has been used to characterise invasive isolates of *S. pneumoniae* in Scotland. We have used this data to study the relationships between invasive pneumococcal isolates and identify clones which may be associated with developing antibiotic resistance and capsule (serotype) switch. Studies of this type are important for better understanding the epidemiology of pneumococcal disease and for informing vaccine policy.

CM 05 Rapid detection of viable *Mycobacterium paratuberculosis* in milk using phage amplification

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Mycobacterium avium subspecies *paratuberculosis* (MAP) is the causative organism of Johne's disease in cattle. Although not

currently classed as a zoonotic agent, a link has been proposed between MAP and the development of Crohn's disease in humans. MAP has been identified in retail samples of pasteurised cow's milk in the UK, causing concern that milk is the vehicle for transmission of MAP to humans.

Detection of MAP by culture is hampered by the slow growth of the organism, the decontamination procedures required to remove competitors and occurrence of MAP in low numbers. We have developed a bacteriophage-based test, which can rapidly and specifically detect low numbers of MAP bacilli in milk. Using this method the detection and enumeration of MAP in spiked milk samples can be completed in 24 hours, rather than the 12 weeks taken by conventional culture methods. In pasteurised milk samples, positive results were only seen if viable MAP were present. The method developed does not require stringent chemical decontamination of the samples and therefore lowers the potential limit of detection of MAP cells that can be detected in milk.

CM 06 Variable membrane proteins in *Borrelia recurrentis*

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Louse-borne relapsing fever (LBRF) caused by the spirochaete *Borrelia recurrentis,* was once a major worldwide cause of morbidity and mortality. The incidence of disease has decreased during the last fifty years however it remains a major pathogen in Ethiopia and surrounding countries, especially during the rainy season. Research on this organism has been hampered in the past as it was unable to be cultivated. We have been able to cultivate this spirochaete from the blood of Ethiopian patients and now are investigating the variable membrane proteins (vmps) of this pathogen. It is likely that these proteins play a pivotal role not only as a means of immune evasion, but may also directly influence the pathology of this disease.

Initial characterisation of these bacteria reveals vmps that fall into two major groups: 34–45 kDa variable large proteins (vlp) and 21–24 kDa variable small proteins (vsp). In order to study these vmps further and deduce a mechanism for their variation, we have selected three isolates for further study. From these, we have produced sub-genomic libraries, and are currently characterising various vmp genes. Initial findings show the existence of many genes with sequence homology with *B. hermsii,* a tick-borne relapsing fever spirochaete in the USA. It would also appear that we have isolated a range of presumptive functional and pseudogenes from the alpha and beta vlp families. We present our current characterisation of these genes and discuss our strategies for determining mechanisms for their variation.

CM 07 Role of biofilms on the failure of implanted speech valves

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Carcinoma of the larynx accounts for approximately 2 % of total malignancies in the UK. Around 10–20% of these patients require a tracheo-oesophageal speech valve which is made of a silastic material. These valves have a limited 'life span', averaging about 3 months and this is due to biofilm formation on the valve. The aim of this study is to understand the mechanisms by which the biofilm causes valve failure *in vitro* and it is hoped that this will enable us to design a better valve for use *in vivo* that can tolerate bacterial and fungal colonisation.

An artificial throat manifold was constructed based on the work of Leunisse *et al.* (*Acta Otolaryngologica.* 1991, 119, 604–608). Briefly, silastic discs were fitted into a glass holder, which was inserted into the manifold, such that the discs were immersed in liquid growth medium. The system was maintained at 37°C and gassed with 5% CO_2 in air. Initially, the medium (PYG) was inoculated with *Candida albicans* , Enterobacteria and Streptococci and the system was left to run for 34 days. During this period, silastic discs were removed and analysed for microbiology and by mechanical testing to look for changes in the properties of the material.

The results to date show that the artificial throat is capable of supporting the growth of biofilms on the silastic disc. Preliminary data on the mechanical properties of the silastic suggest that stiffening of this material occurs over 34 days. Further studies using this system will allow us to generate more data that can be incorporated into a finite element model of the speech valve, thus allowing the behaviour of the valve to be studied as the biofilm develops.

CM 08 Adherence of *B. fragilis* to extracellular matrix components under different redox conditions

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The anaerobic Gram negative bacterium, *Bacteroides fragilis* is an opportunistic pathogen considered to be the most virulent of the *Bacteroides spp.* and is responsible for the majority of clinical infections, which include sepsis, bacteraemia and abscesses. Their pathogenic potential is influenced by the redox conditions in which they are grown. Previous studies have shown that under reducing conditions (mimicking conditions of the commensal state), *B. fragilis* forms a compact aggregation at the HeLa cells than under oxidizing conditions (mimicking conditions of the pathogenic state) when it is more invasiveness. One important step in many bacterial infections is adherence to host cell surfaces. Bacterial agglutination assays using latex beads coated with laminin, fibronectin and fibronectin with heparan sulfate have revealed differences between strains originating from abscesses (ATCC 25285), bacteraemia (ATCC 43859 and 1081) and otitis (MC2), as well as differences between cultures of the same strains grown under both reducing and oxidizing conditions. A panel of monoclonal antibodies specific against polysaccharide surface components of *B. fragilis,* implicated as potential virulence determinants, has identified variation in surface structure and differences in protein expression have also been detected. These findings may help to explain how this bacteria, member of the normal flora, is involved in severe pathogenic processes.

CM 09 *Propionibacterium acnes* serotypes I and II represent two phylogenetically distinct subgroups

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Propionibacterium acnes is a Gram-positive anaerobic coryneform bacterium that forms part of the resident human microbiota. *P. acnes* is now widely acknowledged as an opportunistic pathogen implicated in acnes vulgaris, dental infections, meningitis, osteomyelitis, endocarditis and prosthetic hip infections. Two distinct serotypes of

P. acnes, known as type I and II, exist. These types can be distinguished based on serological agglutination tests, cell wall sugar analysis and monoclonal antibody labelling. To date, the phylogenetic relationship between these phenotypically different organisms has not been investigated. A clearer understanding of the phylogeny of *P. acnes* will be valuable for epidemiological studies and clinical diagnosis of this important opportunistic pathogen.

DNA sequencing of the *P. acnes* 16S gene (1492bp) revealed little difference between serotypes I and II. As the resolving power of rRNA sequences is limited amongst highly related bacteria, especially Gram-positive organisms, we conducted a phylogenetic study of *P. acnes* based on analysis of the *recA* gene (1047bp). On the basis of these studies we now report that *P. acnes* serotypes I and II correspond to phylogenetically distinct subgroups. Further analysis of potential virulence-related gene sequences has confirmed the phylogenetically distinct nature of these serotypes. In other organisms, such as *Burkholderia cepacia* complex species, distinctions in *recA*-based phylogeny, but not 16S rRNA, have correlated with differences at the species level. It remains to be determined if the differences in *P. acnes recA* phylogeny reflect species differences. At present, experiments are under way to develop a DNA-based approach for the identification of *P. acnes* serotypes I and II based on the *recA* gene.

CM 10 Improved identification of *Mycobacterium* cultures, in a diagnostic setting

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Mycobacterium other than tuberculosis (MOTT) have increased in incidence and in clinical significance over the past decade. Clinical disease caused by MOTT is often indistinguishable from that caused by M. tuberculosis. It is therefore important to rapidly accurately identify all mycobacterial species. At the Royal Free Hospital NHS trust we routinely identify our positive Mycobacterium cultures using Becton Dickinson Strand Displacement assays for *M. tuberculosis* complex, *M. avium* complex and *M. kansasii*. Using these assays we are only able to identify 70% of isolates. Though the reference laboratory identifies our isolates, it would be preferable if all isolates were identified in-house in order to decrease turn-around times and improve patient management. In order to address this we have evaluated the INNO-LiPA Mycobacterium Identification kit and the use of a universal bacterial 16S rDNA PCR followed by sequencing. We evaluated the methods for accuracy of results, time to perform and cost per sample. The INNO-LiPA assay was rapid and simple to perform but was expensive and failed to identify two clinical *M. fortuitum* isolates. Although labour intensive, sequencing was less expensive and, potentially enables all isolates of Mycobacterium to be identified.

CM 11 Susceptibility of *Pseudomonas aeruginosa* isolates to antibiotics under aerobic and anaerobic conditions

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Recent evidence indicates that the airway mucus of cystic fibrosis patients is anaerobic and that bacteria, such as *Pseudomonas aeruginosa*, that cause infection in the lungs of these patients are growing under anaerobic conditions. Current antibiotic treatment of such infection which is based on the susceptibility of the infecting bacteria growing aerobically may consequently be inappropriate. The aim of this study was, therefore, to determine the susceptibility of clinical *P. aeruginosa* isolates growing under both aerobic and anaerobic conditions, to antibiotics currently used to treat cystic fibrosis lung infection. The minimum inhibitory concentrations (MICs) of ceftazidime, tobramycin, meropenem, aztreonam, piperacillin and piperacillin/tazobactam, for clinical *P. aeruginosa* isolates were determined using the British Society for Antimicrobial Chemotherapy broth microdilution method. Although there were differences between the MICs for some of the isolates under aerobic and anaerobic conditions, the overall results showed that growth of the isolates anaerobically did not affect their susceptibility to the antibiotics tested. The results suggest, therefore, that MICs determined in routine diagnostic laboratories for aerobically grown *P. aeruginosa* cystic fibrosis isolates can still be used to enable an informed decision to be made regarding treatment of infection caused by these isolates, even if they are growing anaerobically.

CM 12 Rapid colorimetric synergy studies using ceftazidime and tobramycin combinations against *Pseudomonas aeruginosa*

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As a result of the significant increase in the number of antibiotic resistant micro-organisms causing infection, clinicians are now routinely using combination antibiotic therapy with the aim of achieving antibiotic synergy. We have developed a rapid, colorimetric time-kill assay to screen antibiotic combinations for synergistic activity and shown that it produces results consistent with conventional methods in a shorter duration of time. In the present study the *in vitro* activity of ceftazidime and tobramycin against a number of clinical *P. aeruginosa* isolates with varying susceptibilities to both drugs was determined by this colorimetric time-kill assay over a 24 hour period and the results compared with those obtained by conventional viable count methods. Synergy was detected for 50% of strains at 8 hours and for 75% of strains at 24 hours. Results were comparable when measured by both viable count and colorimetric determination. The rapid colorimetric screening of antibiotic combinations can help ensure the earlier commencement of appropriate antibiotic therapy.

CM 13 Development of a rapid colorimetric based antimicrobial susceptibility test for *Pseudomonas aeruginosa*

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Rapid selection and administration of appropriate antibiotics is imperative for the successful resolution of severe and life-threatening infections. Unfortunately, methods current methods to determine the susceptibility of infecting bacteria to antibiotics involve overnight incubation which may result in appropriate antibiotic therapy being delayed. The aim of this project was, therefore, to develop a colorimetric based antimicrobial susceptibility test that would provide rapid results and enable appropriate antibiotic therapy to be commenced more quickly. The Minimum Inhibitory Concentrations (MICs) of ofloxacin, tobramycin, piperacillin, meropenem and ceftazidime, for *Pseudomonas aeruginosa* strains were determined using the broth microdilution method at 18 h and an XTT based colorimetric method at 5 h and the results compared. The MICs of piperacillin, meropenem and ceftazidime could not be determined using the colorimetric assay at 5 h as there was no difference in growth in test and control wells. However, there was excellent

agreement between the MICs for ofloxacin and tobramycin determined after 5 h using the colorimetric assay and after 18 h using conventional methods. The data suggests that a colorimetric based antimicrobial susceptibility test could provide a useful method for determining the susceptibility of *P. aeruginosa* to bactericidal antibiotics.

CM 14 Comparison of DNA extraction methods for recovery of methicillin-resistant *Staphylococcus aureus* DNA from broth culture and human sera

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DNA amplification techniques have a central role in diagnosis of infection from clinical specimens. Due to low abundance of DNA in samples and inhibition of amplification, optimisation of DNA recovery is critical.

This study aimed to obtain a procedure that is efficient, reproducible, rapid, simple, economic, and requires low volumes of serum.

Five DNA extraction methods (GES, phenol, guanidine thiocyanate/SDS, and kits from Promega and Qiagen) were tested for the ability to recover MRSA DNA from broth. Qiagen, phenol, and Promega methods extracted DNA successfully as demonstrated by PCR and spectrophotometer readings. These three methods and two other kits [Dynabeads (Dynal) and Gene Releaser (Cambio)] were used to extract DNA from human serum seeded with MRSA. Qiagen, Dynabeads, and Gene Releaser methods eliminated PCR inhibitors most effectively. Dynabeads and Gene Releaser were more sensitive (minimum detection 200cells/ml of serum) and used the least serum. But the Qiagen kit was more reproducible. In conclusion, no one method was found to be ideal in all aspects. However, the Qiagen kit was found to give more favourable results than other methods used in respect of reproducibility, reducing the effects of PCR inhibitors and cost, and gave acceptable sensitivity (minimum detection 300cells/mlof serum) and processing time.

CM 15 Cloning of the multiple banded antigen of *Ureaplasma* spp. for the development of an ELISA

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Ureaplasma spp. (14 serotypes) can be related to adverse pregnancy outcome. Development of serological assays for *Ureaplasma spp.* antibodies has been hampered by the low purity of antigen used in the assays. The aim of the present study is to produce recombinant antigens for all serotypes for the development of a serological assay. The multiple banded antigen (MBA) of *Ureaplasma spp.* is the major surface antigen recognized during infection. This makes it a suitable candidate for use in serological assays. The MBA gene from the serotypes were amplified and cloned into a pTrc-His Topo plasmid. The expressed proteins, containing a poly-His tag, were purified and analysed by western blot assay using an anti-poly His antibody and by ELISA using the 14 serotype-specific monoclonal antibodies (MAbs). Except for serotypes 1, 6 and 13, the MBA was successfully expressed for all serotypes. MBAs of serotypes 2, 3, 4, 5, 8, 9, 10, 12 and 14 were recognised by serotype specific MAbs in ELISA. These results indicate that the purified recombinant multiple banded antigens are promising for de detection of antibodies against *Ureaplasma spp.* serotypes.

CM 16 Measuring the activity of resuscitation promoting factors using ATP bioluminescence

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Resuscitation promoting factors (Rpfs) are growth factors, produced by a range of Gram-positive bacteria, including *Mycobacterium tuberculosis*. They promote the growth of dormant organisms. A better understanding of Rpfs might shed light on the phenomenon of dormancy in *Mycobacterium tuberculosis*. Research into Rpfs has been hampered by the fact that they are unstable, losing activity within days of preparation. Furthermore, assays for Rpf activity take a number of days to perform during which time the proteins become inactive. To overcome this problem a rapid assay was developed, based upon the measurement of intracellular ATP in cultures of *Micrococcus luteus*. Using this assay concentrations of ATP up to 65 times greater were found in cultures exposed to Rpf compared to those with no Rpf. Availability of a convenient assay for Rpf activity will be of great value in studying these proteins further.

CM 17 Isolation of *Balamuthia mandrillaris* from the brain biopsy and the cerebrospinal fluid of a granulomatous amoebic meningoencephalitis patient using human brain microvascular endothelial cells

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Here, we report for the first time, the isolation of *Balamuthia mandrillaris* from a granulomatous amoebic meningoencephalitis (GAE) patient in the United Kingdom using primary human brain microvascular endothelial cell cultures. *Balamuthia mandrillaris* were isolated both from the brain tissue as well as the cerebrospinal fluid (CSF) specimens. This is the first report describing the isolation of *B. mandrillaris* from the CSF sample of a GAE patient. Clinical specimens were incubated with the human brain microvascular endothelial cells for upto 45 days. Initially, *B. mandrillaris* were identified based on morphology with characteristic pseudopodia and were later confirmed using polymerase chain reactions using *Balamuthia*-specific primers. Use of chelex for DNA extraction was successfully employed for the sensitive detection of *B. mandrillaris*. Additionally, host serum reacted strongly with *B. mandrillaris* antigens from baboon (1:10 000) in the indirect immunofluorescence assays, further confirming *B. mandrillaris* as the causative agent. The polymerase chain reaction assays using the CSF specimen together with the indirect immunofluorescence assays should help in the rapid and sensitive identification of *B. mandrillaris*, which may help a favourable prognosis. Role of human brain microvascular endothelial cells as a potential model for the blood-brain barrier and its use to understand the pathogenesis and pathophysiology of *Balamuthia* meningoencephalitis will be crucial to develop therapeutic interventions.

CM 18 Re-emergence of early pandemic *Staphylococcus aureus* as a community-acquired methicillin-resistant clone: association of Panton–Valentine leukocidin with enhanced virulence

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During the 1950s, penicillin-resistant *Staphylococcus aureus* of phage type 80/81 became pandemic causing serious hospital- and community-onset infections. This clone was strikingly virulent as, unusually for *S. aureus*, it caused serious invasive disease in healthy children and young adults. Phage type 80/81 was largely eliminated in the 1960s coincidental with widespread use of penicillinase-resistant B-lactams. Here we show that early phage type 80/81 isolates possessed Panton–Valentine Leukocidin, associated with increased virulence in children and young adults. Multilocus sequence analysis shows that descendants of phage type 80/81 have acquired methicillin resistance and are emerging as a cause of community-onset MRSA disease in several countries.

CM 19 Effect of nutrient limitation on the production of *Bacillus cereus* haemolysin BL toxin

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Bacillus cereus can cause two types of food poisoning syndromes due to the production of the emetic toxin cereulide or a variety of enterotoxins. Four types of enterotoxin have been characterized and the production one of the protein complexes, hemolysin BL (HBL), was investigated in this study. A chemically defined medium for the growth of *B. cereus* was established, enabling cells to be grown under growth-limiting concentrations of various nutrients. Our results show that different toxin profiles and levels are seen when *B. cereus* is grown in either complete medium (oxygen-limited), phosphate-limited or magnesium-limited medium. Complete medium gave approximately 30-fold increase in HBL compared to either phosphate- or magnesium-limitation of growth. We have also shown that these levels are increased by the addition of protease inhibitors to the cultures.

CM 20 The distribution of chromosomal mutations in drug resistance genes

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Bacterial cells have a multiplicity of coping mechanisms to deal with external stresses. Survival within the macrophage is crucial for *M. tuberculosis* to persist and cause infection. It is within this environment that bacteria encounter oxidative stress. Due to oxidative stress, mutations can be induced by the oxidation of guanine residues in DNA, causing a higher incidence of G→A mutations then would be expected. In other bacterial species, oxidation of cytosine resulted in a high incidence of C→T mutations. We have reviewed the percentage of base pair mutations that occur in rifampicin resistant strains. C→T substitutions in the *rpoB* gene, conferring resistance to rifampicin, occur most frequently (66%) whereas G→A mutations occur at 0.34%. This dramatic difference in% may be because only a limited number of mutations conferring resistance are sufficiently fit to permit survival and this disproportion is merely a chance phenomenon.

CM 21 A novel mutation in the quinolone resistance determining region of *parC* gene in *S. pneumoniae*

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Fluoroquinolones are increasingly being used to treat lower respiratory tract infections for which *S. pneumoniae* is one of the most important pathogens especially in penicillin resistant or intolerant cases. Quinolone resistance in *S. pneumoniae* is mainly conferred by mutations in the quinolone resistance determining region (QRDR) of DNA gyrase genes *gyrA* and *gyrB* and Topoisomerase IV genes parC and parE.

Common mutations in laboratory generated resistant strains for *parC* are S79Y and D83Y and for *gyrA* S81Y and S81F. The QRDR regions of *parC* and *gyrA* from 47 clinical isolates from Taiwan and USA were sequenced and revealed two new mutations. These were K137N and R95C for *parC* and D80H for *gyrA* and were associated with increased MICs for ciprofloxacin and moxifloxacin.

These results suggest that mutations identified in clinical isolates may not be the same as those from laboratory derived resistant mutations.

CM 22 Population genetics of penicillin intermediate resistant isolates of *Streptococcus pneumoniae* from a semi-closed community in northern Tanzania

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Penicillin resistance in *S. pneumoniae* is increasing globally and is thought to be dominated by the spread of successful clones.

Penicillin intermediate resistant isolates were collected from a semi-closed community in northern Tanzania during a prospective study on the affect of community treatment with Azithromycin for Trachoma. Out of 382 samples collected 69 were intermediate for penicillin and the remaining 313 were sensitive. PBP2B and PBP2X PCRs were performed for the penicillin intermediate strains and the amplimers digested with *Hinf*I and *Dde*I. Using Bionumerics the degree of clustering was calculated for the four digestions and mean values were 74%, 25%, 29% and 22% for PBP2B *Hinf*I, PBP2B *Dde*I, PBP2X *Hinf*I and PBP2X *Dde*I respectively. Using PBP genotype and serotype almost all intermediate resistant strains were distinct.

These data suggest a highly polymictic population with respect to PBP genotypes and that resistance to penicillin is evolving locally. This is in contrast to the common assumption that recent emergence of penicillin resistance is frequently the result of donation of partial PBP genes from already resistant isolates and the spread of internationally recognised multi-drug resistant strains is facilitating and accelerating this. This community may potentially offer an opportunity for studying the evolution of penicillin resistance in *S. pneumoniae* as it emerges independently of fully penicillin resistant strains.

CM 23 Sub-species typing of clinical *Staphylococcus epidermidis* isolates using denaturing gradient gel electrophoresis analysis of the 16S–23S internal transcribed spacer

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Staphylococcus epidermidis is one of the major pathogens responsible for prosthetic joint infections and the typing of *S. epidermidis* could be useful to understand the epidemiology of this organism. Denaturing gradient gel electrophoresis (DGGE), a powerful tool to detect single-base pair differences was applied to the 16S–23S internal transcribed spacer (ITS) to evaluate the method as a sub-species typing technique of clinical *S. epidermidis* strains.

All clinical *S. epidermidis* isolates, recovered during prosthetic joint surgeries, were identified with conventional methods and ID 32 STAPH. Genomic DNA was isolated and ITS were amplified using the primers with a GC-clamp. ITS-PCR products were run on parallel denaturing gels. After electrophoresis the gels were stained with SYBR gold and visualized on a UV transilluminator.

All samples yielded the same banding pattern following standard agarose gel electrophoresis. In contrast, DGGE of the ITSs yieled more bands which separated into a number of different banding patterns. The ITSs of strains from the same patient showed identical banding patterns, suggesting good reproducibility.

Our results suggest that DGGE analysis of 16S–23S ITS could be an effective method for discrimination of clinical *S. epidermidis* strains.

CM 24 A Scid mouse model For EBV-associated post-transplant lymphoproliferative disease

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EBV-specific immune control is primarily mediated by cytotoxic T lymphocytes (CTL). CTL immunity is reduced in immunosuppressed organ transplant recipients, which may lead to EBV-driven proliferation of virus-infected B lymphocytes and, ultimately, post-transplant lymphoproliferative disease (PTLD). PTLD arises in up to 10% of organ graft recipients, is aggressive and difficult to treat with >50% fatality. Our laboratory collaborates with UK organ transplant centres to study the effectiveness of *ex vivo*-expanded CTL against PTLD.

We use the humanised scid mouse to model PTLD. In this model, sc-inoculated B lymphoblastoid cell lines regularly give rise to PTLD-like tumours that can be directly measured and thus monitored for the effectiveness of novel therapeutic strategies. Using this model, we have demonstrated tumour regression following administration of autologous EBV-specific polyclonal CTL. We have extended these studies to include allogeneic polyclonal CTL as well as virus peptide-stimulated monospecific CTL, peptide antigen-specific CTL clones and NK cells in order to examine our hypothesis that these cells are superior to autologous polyclonal CTL in mediating PTLD tumour rejection. We will present results of these translational *in vivo* studies and discuss the implications for future immunotherapeutic approaches to the disease.

CM 25 Penicillin resistance in Group G β-haemolytic streptococcal infection – the eagle effect revisited – a case report

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Severe invasive β-haemolytic streptococcal cellulitis requires prompt diagnosis and rapid administration of appropriate antibiotic therapy. Because group A streptococcus continues to be exquisitely sensitive to β-lactam antibiotics, clinical studies recommend the intravenous administration of Penicillin G [1]. Despite known antibiotic sensitivity to penicillin however, there have been some treatment failures reported. Known as the 'Eagle Effect' [2], penicillin appears effective against group-A streptococcus if given early in the infective process or where streptococcal titres are low. If however the infective process is allowed to progress or inoculum size is high, penicillin shows reduced efficacy (also known as the Inoculum effect [3]).

Although well documented with group-A streptococci, the Eagle Effect has been less well reported with other strains of streptococcal infection. In this report, we examine a case of group G β-haemolytic streptococcal cellulites in a healthy 47 year old male in which treatment failure with penicillin was observed despite known culture sensitivity. This finding may implicate the Eagle Effect in poorly responding group G β-haemolytic infections and may have important implications for clinical management.

CM 26 Transfer of doxycycline resistance among oral bacteria *in vivo*

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There is concern that the administration of systemic antimicrobials for non-life-threatening conditions such as gum disease may increase the numbers of resistant bacteria in the mouth and that resistance may be transferred to pathogens. The aim of this study was to determine if increased resistance following doxycycline treatment resulted from clonal expansion or transfer of genes encoding resistance determinants. Subgingival plaque samples were collected from 2 patients with periodontitis before and after 7 days systemic treatment with doxycycline. Samples were plated onto TYC medium with and without doxycycline. 200 streptococcal isolates were subcultured before treatment and all resistant streptococci post-treatment. Isolates were identified by sodA gene sequence analysis and genotyped by Rep-PCR. In patient 1, the resistant isolates post-treatment were found to be identical to resistant strains present pre-treatment. However, in patient 2, a clone sensitive to doxycycline pre-treatment was found to be resistant post-treatment. The molecular basis for this change in phenotype is currently under investigation.

In conclusion, the results of this study suggest that the increase in resistance observed during systemic treatment with doxycycline is the result of both the expansion of resistant clones as well as, potentially, the transfer of resistance determinants to sensitive bacteria.

CM 27 TB Diagnosis using the Becton Dickinson ProbeTec SDA System: what do the results actually mean?

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During this study a prospective evaluation of the BD ProbeTec SDA System for the diagnosis of pulmonary and non pulmonary tuberculosis was performed. A total of 744 samples were analysed from patients with clinically suspected TB.

Using culture as the gold standard the sensitivity and specificity for pulmonary samples were 98% and 88% while for the non pulmonary samples the sensitivity and specificity was 60% and 94%, respectively. There is strong evidence to suggest the presence of a genuine 'grey zone' within the SDA values. Within this area 'SDA positives' do not always correlate with positive smear and/or culture results. These findings support the possible introduction of several reporting categories which would be based on the likelihood that a positive SDA result is obtained from a sample from a patient with clinical TB.

CM 28 A PCR-based comparative survey of arthropod-transmitted infections in ticks parasitising companion dogs and cats in southern France

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There is minimal information on pathogen carriage by ticks parasitising companion animals. Ticks were collected over a 6 months period from 89 dogs and 51 cats presented to veterinary clinics in southern France. After collection into ethanol, ticks were morphologically identified, DNA extracted and samples screened by PCR analysis for genera *Babesia, Ehrlichia, Anaplasma* and *Borrelia*. Positive samples were sequenced. Of the 294 ticks analysed, 51 (17%) were PCR positive; 29 for *Ehrlichia*, 17 for *Babesia,* 2 for *Hepatozoon* sp and 3 were co-infected. Only 3/51 PCR positive ticks were feeding on animals PCR positive for the same organism. No PCR positive ticks were identified on cats. On dogs, *Dermacentor* species ticks carried *Babesia canis canis* alone (6.5% positive). *Rhipicephalus sanguineus* ticks carried *Ehrlichia canis* (15.7%), *Babesia canis vogeli* (5%) and *Hepatozoon canis* (2%) with 2 co-infections. *Ixodes* species ticks carried a tick endobiont *Ehrlichia* species (12.3%) and a *Babesia odocoilei*-like organism (6.2%) with one co-infection. The infection challenge for dogs is high for the major pathogens *Babesia canis canis* and *Ehrlichia canis*. *Ixodes* species ticks parasitising dogs also carried a *Babesia* species closely related to the human pathogen EU1 recently isolated from a symptomatic human case in Austria.

CM 29 Tick-transmitted infections in New Caledonian dogs: a new niche for *Rickettsia honei*?

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The native fauna and flora of New Caledonia have evolved in isolation and the native dog population (30,000 animals) is effectively closed due to minimal import /export of animals. EDTA blood samples were taken from a convenience sample of pet dogs (n=127) from 5 veterinary clinics in Noumea, Païta and Bourail. Ticks (n=204) found on these dogs were collected into ethanol and identified morphologically. DNA was extracted from blood samples and screened by genus-specific PCR analysis for *Babesia*, *Ehrlichia, Anaplasma* and *Rickettsia* species. Adult ticks (n=177) were PCR screened using a degenerate assay for *Babesia* and *Hepatozoon* apicomplexan species, and genus-specific PCR analysis for *Babesia*, *Ehrlichia, Anaplasma* and *Rickettsia* species. Positive samples were analysed using DNA sequencing. Of the blood samples, 3/127 were positive for *Anaplasma platys* but negative for DNA of other organisms. All ticks were identified as *Rhipicephalus sanguineus;* 18/177 (10%) infesting 12 dogs were positive for *Anaplasma platys* and 18/177 (10%) from 7 dogs were positive for *Hepatozoon canis* DNA. In addition, 7/177 (4%) from 2 dogs were positive for *Rickettsia honei,* a recently characterised spotted fever group organism. The presence of *R. honei* in ticks parasitising companion dogs suggests that it may also be present in the human population of New Caledonia.

Posters

Environmental & Microbiology Group

EM 01 Fungal degradation of calcium-, lead- and silicon-containing minerals

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Microbial weathering mechanisms involve processes such as corrosion, mineral dissolution, mineralogical modification via transformation into secondary minerals and, in the case of fungi, penetration into the mineral substrate by hyphae. Corrosion, dissolution, and mineral transformations often occur as a result of physico-chemical interactions with fungal metabolites, e.g. H^+ and organic acids. The objective of this work was to investigate the influence of a carbon source (glucose) on the weathering abilities of three organic acid-producing fungi (*Aspergillus niger*, *Serpula himantioides* and *Trametes (Coriolus) versicolor*) towards apatite ($Ca_5(PO_4)_3F$), galena (PbS) and obsidian (SiO_2). Our results show that while the fungi were capable of mineral surface colonization under low nutrient conditions, the extent to which the effects of such colonization were manifest, especially the corrosion of mineral surfaces and secondary mineral formation, were affected by the availability of a carbon source. Only *S. himantioides* and *T. versicolor* were able to corrode the surface of apatite in the absence of glucose but none of the fungi were capable of doing so with the other minerals. With galena, secondary mineral and precipitate formation was entirely dependent on the availability of glucose. Nutritional influence on biogeochemical activities of fungi may clearly affect element cycling in the environment, although it is also evident some degree of geochemical change may result under nutrient-limited conditions.

EM 02 Rock and mould: transformation of carbonate minerals by fungi

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Fungi promote rock weathering and contribute to the dissolution of mineral aggregates in soil through the excretion of H^+, organic acids and other ligands, or through redox attack of constituents such as Mn and Fe. Fungi can also play a role in mineral formation through the precipitation of secondary minerals, e.g. oxalates, and through the nucleation of crystalline material onto cell walls, resulting in formation of biogenic micro-fabrics in mineral substrates. Such interactions between fungi and minerals are of importance to biogeochemical cycles including those of C, N, S and P. Our research has demonstrated that fungi can play an important role in the development of micro-fabrics in limestone ($CaCO_3$) and dolomite ($CaMg(CO_3)_2$). Scanning electron microscopy and energy-dispersive X-ray micro-analysis of rehydrated limestone samples has revealed the presence of mineralized fungal filaments. X-ray diffraction analysis has indicated that these were probably secondary carbonates. Other experiments using limestone isolates and laboratory cultures grown in microcosms amended with calcium carbonate showed that fungi precipitated secondary crystals, exhibiting a range of different morphologies, and identified as a mixture of calcite ($CaCO_3$) and whewellite (calcium oxalate monohydrate, $CaC_2O_4.H_2O$).

EM 03 Molecular methods to assess the diversity of rock fungal communities

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Fungi play important, though unappreciated, roles in metal–mineral biogeochemistry though a significant barrier to our understanding is the lack of accurate means of characterizing natural populations in rocks and minerals. Establishing the diversity of rock-dwelling fungi by applying molecular techniques based on total DNA extraction would provide a desirable tool to improve our knowledge of geomycology. The objective of this research was therefore to apply molecular techniques to the characterization of fungal community composition, therefore eliminating the problems associated with traditional isolation and culture. Firstly, a successful method for extracting amplifiable DNA from a selection of sandstone and limestone was developed to permit further genetic analysis. This enabled application of the automated ribosomal intergenic spacer analysis (ARISA) technique which exploits the length heterogeneity of the internal transcribed spacer (ITS) region of the rDNA. Another fingerprinting method used was denaturing gradient gel electrophoresis (DGGE) which is based on the 18S region of the rDNA. Both techniques resulted in the generation of genetic community fingerprints of endolithic fungal communities. Our results have shown that fungal communities in sandstone and limestone are relatively diverse for such apparently extreme and inhospitable environments. Furthermore, community composition varied greatly between different geographical locations and rock type, revealing that rock substrates support a richer fungal diversity than previously thought. We can conclude that molecular methods provide a powerful technique for analyzing rock-inhabiting fungal communities and therefore their roles in biogeochemical processes.

EM 04 Cryo-scanning electron microscopy of ectomycorrhizal roots

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Ectomycorrhizas are symbiotic associations between fungi and the roots of host plants where the fungus forms a mantle of fungal material around the root tip and penetrates the intercellular space between cortical root cells forming the 'Hartig net'. A variety of conventional light and electron microscopy methods are currently used to study features of the ectomycorrhizal symbiosis, all with advantages and disadvantages depending on the research context. The aim of this research was to investigate the applicability of Cryo-SEM to the study of ectomycorrhizal root morphology, and involved cryo-preparation and fracturing of fungal-plant specimens. If successful, this technique may prove useful for examination of metal-mineral transformations in the mycorrhizosphere environment. Samples were examined using an Hitachi S-4700 field emission gun scanning electron microscope (FEG SEM) operating at an accelerating voltage of 5kV. Cryo-SEM of cross-fractured mycorrhizal tips showed that a mantle and a well-defined Hartig net were present almost to

the root endodermis. Cryo-SEM allowed observation of both interior and exterior morphology and appeared to preserve the complex structure of ectomycorrhizal roots better than some other microscopic techniques.

EM 05 Scanning electron microscopy of fungal–mineral transformations

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The ability of fungi to interact with minerals, metal(loid)s and organic compounds through biomechanical and biochemical processes, makes them ideally suited as biological weathering agents of rocks, minerals and building stone. The overall aim of present research is to elucidate the role of fungi in geomicrobiological processes, emphasising their deteriorative potential on rock and involvement in the formation of secondary mycogenic minerals. In this study, scanning electron microscopy (SEM)-based techniques were used to study metal-mineral transformations by fungi. We have found that environmental SEM in wet mode is particularly applicable for observing such interactions in their natural microenvironment with coupled X-ray element mapping revealing sequestration and localization of metals associated with the biomass and as constituents of secondary materials. Cryo-SEM enabled observation of both interior and exterior microstructures and provided structural information on the formation of secondary mycogenic minerals on fungal biomass.

EM 06 Interactions of ectomycorrhizal fungi with saprotrophic and root pathogenic fungi: effects on carbon and mineral nutrient allocation

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Our recent studies of mycelial systems in soil microcosms using combined image analysis, radioisotope tracers and digital autoradiography have shown that foraging mycelial systems of wood decomposers and ectomycorrhizal fungi interact strongly when grown from natural carbon sources in non-sterile soil in microcosms. Mineral nutrient foraging and transport by interacting saprotrophic and ectomycorrhizal mycelia, together with the patterns of carbon allocation from plants to mycorrhizal mycelia, have been visualised and quantified using radioisotope tracers. ^{15}N allocation has been determined using destructive sampling followed by mass spectrometry.

Mycelial systems of *Paxillus involutus* resisted invasive cords of *Phanerochaete velutina*, but led to allocation of host-derived carbon away from the interaction zone. However, *Suillus bovinus* had reduced allocation of host derived carbon to the entire extraradical mycelium when over grown by *Ph. velutina* mycelia, compared to controls. In contrast greater ^{15}N was allocated to cords of *Ph. velutina* interacting with *P. involutus* extraradical mycelia than to non-interactive controls.

Using these techniques we are currently investigating the mechanisms by which extraradical ectomycorrhizal mycelia may also provide protection from migratory and non-migratory root pathogenic fungi. We hypothesise that differences in extraradical mycelial morphology will determine degree of protection of host roots from pathogenic fungal infection.

EM 07 Comparison of biodegradative properties of members of the genus *Rhodococcus* with their Amplified Ribosomal DNA Restriction Analysis (ARDRA) profiles

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Species from the genus *Rhodococcus* are known to be valuable in the bioremediation of contaminated soils and industrial wastewaters. They are frequently isolated from areas polluted with chemicals. It has been suggested that glucose enhances biodegradative abilities in these species. ARDRA is comparable to other methods and useful for the discrimination of species. In this study, a selection of isolates held in the culture collection at the University of Bradford were assessed for their ability to degrade a range of potential environmental pollutants both with and without glucose. Many of the isolates were processed to provide profiles using ARDRA.

Isolates showing the wider range of biodegradative properties produced ARDRA profiles consistent with the species *R. erythropolis* or *R. globerulus*. Enhancement of activity by glucose was confirmed.

Isolates shown to have the widest activity were confirmed as the most active for chemical pollutants such as phenol, engine oil and mineralised methylated spirits, although some had been isolated from river sediments not known to be contaminated. Species designation rather than the source of the organism determines their activity. ARDRA, which is cheaper than sequencing and can be done without expensive equipment, discriminated effectively between the two species, *R. erythropolis* and *R. globerulus*.

EM 08 Fungal proteases for unhairing cattle hides in leather manufacture

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Leather production involves the use of large quantities of noxious chemicals, particularly in the early stages of converting animal hides to tanned leather. Removal of hair from hides by sulfides is a process that could be improved by enzyme technology, as sulfide digests the hair but does not remove residues in hair follicles, which can be visible in the final processed leather. (Sulfide presents a major effluent discharge of the industry.)

Fungal proteases with a range of proteolytic activities offer a potential solution to unhairing hides that will remove hair completely from the follicles. (This would provide a new option of 'hair-save' for the industry.) One problem encountered is that the specific protein composition of the anchoring matrix of the hairs in the follicles is not fully understood, so identifying specific enzyme activities is not optimal. Activities such as collagenase, elastase, and keratinase are potential candidates for unhairing, but enzyme assays to assess activities are not necessarily appropriate to the task as the assay substrates are modified from natural proteins in skin.

A matrix will be presented of enzyme activities of proteases produced by fungi that have differing combinations of enzyme activities on substrates such as hide powder-black, azocoll, collagen 1, elastin-Congo red, and keratin-azure. These activities will be correlated with unhairing ability of the enzymes.

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EM 09 The application of a high through put analysis for the screening of potential biosurfactant-producing micro-organisms from natural sources

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Biosurfactants have recently been shown to have therapeutic effects in the treatment of diseases caused by certain viruses. This has stimulated the drive to discover new sources of biosurfactant. Biosurfactant activity can be evaluated by emulsification tests, surface tension measurement and the determination of various physical-chemical properties, but there are limited possibilities to automate these methods so as to screen large numbers of samples. Accordingly, this paper describes a high throughput-screening assay, intended to aid in the identification of biosurfactants produced from natural sources. This method is based on the effect of meniscus shape on the intensity of transmitted light, when liquid samples are illuminated in a 96-well plate. Compared with previous tests based on emulsification or surface tension, the assay was found to be more sensitive, rapid and easier to perform but does not need specialized equipment or chemicals. In the present study, the most promising strain was selected from thirty-six different isolates originating from land contaminated by aviation fuel. The biosurfactant produced by this bacterium was found to be able to reduce the surface tension of pure water from 72 to 28.75 mN m^{-1}. The results have demonstrated that the method proposed is an efficient and effective screening procedure, which excludes the bias that results from the surfactant properties of medium used for bacterial growth.

EM 10 Biosynthesis of arsenobetaine involving bacterial formation of an arsenic–carbon bond

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Arsenobetaine [$(CH_3)_3As^+CH_2COO^-$] has been shown to be the major arsenical in almost all-marine animals examined and, through human consumption of seafood, is the major arsenical compound in the diets of most people. Anaerobic microbial activity converts algal dimethylarsinoylribosides to yield compounds with a C2 chain attached to arsenic (dimethylarsinoylethanol or dimethylarsinoylacetate), which are considered potential precursors of arsenobetaine. However, fish when supplied with these compounds do not accumulate the arsenic as arsenocholine or arsenobetaine. Is there a bacterial route to arsenobetaine?

Whole-cells and lysed-cell extracts of a *Pseudomonas* sp. were shown to catalyse bioconversion of dimethylarsinoylacetate to arsenobetaine and dimethylarsinate. The universal methyl donor *S*-adenosylmethionine promoted both the rate and extent of arsenobetaine formation. These findings show that the proposed biosynthesis of arsenobetaine from a dimethylarsinoyl-precursor is feasible and suggest that oxidation of dimethylarsinoylethanol would precede the reduction and methylation at the arsenic atom. The presence of such enzymatic activity in a bacterial isolate from marine mussel (*Mylitus edulis*), highlights a possible direct involvement of prokaryotic organisms in the accumulation of organoarsenic compounds within marine animals.

EM 11 The effect of *Chalara longipes* (Preuss) Cooke on decomposition of humic acids from spruce needle litter

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The effect of saprotrophic ascomycete *Chalara longipes* (Preuss) Cooke on humic acids was determined in a cultivation experiment. The fungus was incubated in liquid cultures in a full strength (F systems) and in an organic nitrogen-free medium (F-N systems), both amended with pure humic acids isolated from spruce forest litter. Fungal biomass production and utilization of humic acids was determined. Fungal biomass production was highest in the F systems with humic acids and lowest in the F-N systems and the effect of organic nitrogen and humic acid was significant ($p<0.05$). The presence of organic nitrogen seems to be essential for the growth of *C. longipes*. The fungus utilized the humic acids and molecular weight distribution in media obtained by gel-permeation chromatography showed decrease in the humic acids fraction. Decolourisation of the media reached 75% and 64% in the F and F-N systems respectively. The molecular weight of humic acids probably also decreased as estimated from the increase in the A_4/A_6 ratio. It is supposed that the humic acids utilization by *C. longipes* may be performed due to its organic acids and oxidative enzymes production.

EM 12 Investigation of the Piezotolerant microbial community from a temperate estuarine environment: implications for the biosynthesis of natural products

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Over the past two decades, a wide range of microbial taxa have been isolated from deep-sea trenches. In this environment, temperature, pH, redox state, and nutrient availability change dramatically. As a result, microbes isolated from this micro niche must be able to exhibit some degree of resistance to these physiological parameters. Paradoxically, some extremophilic bacteria are isolated from areas generally considered not to be their ideal environment. This may suggest that they maybe transient at the place of capture, within these deep-sea trenches and not actively growing. These observations raise interesting questions about the inherent ability of 'non' extremophilic bacteria to adapt to these stresses. Recently, molecular phylogenetic approaches studying the 16S rDNA gene have been used to examine the diversity of different hydrothermal communities worldwide. However, the microbial community structure of facultative piezotolerant mesophiles, to our knowledge, has never before been studied in detail. In this study, the piezotolerance of strains isolated from open seawater and seaweed surfaces on the South-East coast of Scotland was investigated. On the basis of this data, the relationship between environmental conditions and piezotolerance will be discussed.

EM 13 A universal method to analyse the diversity of mycosporines and mycosporine like amino acids (MAA) in pro- and eukaryotic micro-organisms and environmental rock inhabiting microbial communities

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Mycosporines and mycosporine-like amino acids (MAA) are frequent protective substances in pro- and eukaryotic micro-organisms. The

major function of these low molecular UV-B absorbing hydrophilic compounds is their capacity to act as photoprotective UV Filters (in prokaryotes) or to exercise a regulatory effect on sporulation (in eukaryotes). Furthermore they can serve as antioxidants and/or have osmoprotective function. Pure cultures of diverse micro-organisms have been studied. The organisms under investigation were eukaryotic fungi (ascomycetes, basidiomycetes, deuteromycetes) and prokaryotes (actinomycetes of the Geodermatophilus genus and different cyanobacteria), which were screened for the content and diversity of mycosporines and MAAs. Furthermore natural stone samples like marble and various sandstones were extracted and the UV-B absorbing compounds were characterized. After sensitive extraction of the water soluble contents reversed-phase HPLC coupled with mass spectroscopy was used to quantify and identify the UV-B absorbing compounds. In total five MAAs, eight mycosporines and one UV-absorbing substance which is yet unknown were successfully extracted and separated by the method suggested. It is stated, that chemically highly unstable mycosporines and MAA on the rock surface are protected either by the presence of living cells or by physical and chemical interactions with the rock substrate itself.

Posters

Education & Training Group

ET 01 Microbiological quality manual for a new food product

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As part of a final year module in practical food microbiology, we have, for several years, run an exercise which we hope gives students greater scope for initiative and originality than more conventional practical exercises.

Students are given a simple recipe for a food product. They then have to search the literature for information on the microbiology of the ingredients, the product and any critical processing steps to identify potential microbiological hazards. In the laboratory they make the product and perform microbiological tests on the ingredients, product in process and final product. Typically they would then determine the shelf life at chill and abuse temperatures and superficially characterise the spoilage microflora. Having decided on pathogens which might pose a threat to consumers of the product, they perform challenge trial(s) to determine the potential for growth or survival of the organisms in the product. They can compare their data with the results from predictive microbiology software such as the Pathogen Modelling Program and Food Micromodel. If they have time or initative, they may also investigate the microbiological effects of changes to the recipe such as decreased salt or increased pH. Throughout the exercise the students have to organise their own work; media and materials are supplied but only at their request, necessitating some thought and careful planning.

At the end of the exercise the students make a short presentation of their work to the rest of the group and submit a written account, both of which are assessed.

This has proved a popular exercise with students, most of whom find it stimulating and challenging, albeit quite hard work.

ET 02 Discovery of bioactive metabolites in microbial cultures: a student practical exercise in industrial microbiology

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The exercise compares the requirements of a screen, based on positive selection pressure, for cellulase-producing microbial isolates with those of a neutral screen for antibiotic producers. The distributions of microbial genera within a series of soil samples, gathered by students from around the campus, are assessed in terms of the diversity of bioactive products secreted by the isolates in each sample.

An initial 'high-throughput' semi-automated screen, based on agar droplet culture, detects, Gram-positive, broad spectrum and antifungal antibiotics in addition to β-lactamase inhibitors, putative β-lactams, and inhibitors of a viral promotor (via the *gfp* reporter gene). This is followed by liquid culture designed to assess the effect of various growth-limiting nutrients on product diversity.

Transferable skills include mass data reduction in addition to manipulative acumen.

ET 03 The production of worksheets to accompany the video series 'Intimate Strangers: Unseen life on Earth'

Katie Murcott, G. Craig & J. Verran

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In 1999, to celebrate its centenary, the American Society for Microbiology produced a 12 x 30 minute video series entitled 'Intimate Strangers: Unseen Life on Earth'. The videos are intended for FE/early years undergraduate education. Aspects of applied and basic microbiology are addressed; eminent scientists describe their work; and a wide range of case studies are also included into the series.

Unfortunately, these videos were not widely distributed, and were only produced in US format. As time progresses, this useful resource will date, with use diminishing, if sufficient relevant and accessible learning activities are not produced to accompany them.

Funding from the MMU Faculty of Science and Engineering and LTSN Bioscience enabled the summer employment (2003) of an undergraduate (the presenter) to evaluate the series, identify particularly useful video segments, and develop accompanying learning activities for use with first year undergraduate classes at MMU. Subsequently, clips will be selected for dissemination via the ASM website (subject to review and approval), to accompany those items already present. Appropriate activities will accompany these clips to enable more active learning.

This poster will report on the use of the videos and activity sheets, and provide evaluation of the video content from undergraduate students.

ET 04 'An Introduction to Practical Microbiology': a DVD package produced by staff and students at Manchester Metropolitan University

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In 1991, a group of students under the supervision of the presenter, produced a video entitled 'An Introduction to Practical Microbiology'. The 30 minute film, accompanied by a 36-page copyright waived booklet, proved successful, and was sold to schools and universities across the UK, and beyond.

As stock of the video diminished, and time progressed, the need for an updated version became apparent. In 2002, a group of students, again under the supervision of the presenter, began to repeat the process. In 2003, a DVD was produced, incorporating a downloadable 36 page colour pdf document. The 30 minute film describes basic laboratory skills in microbiology, which can also be shown as chapters selected from the menu.

The DVD will be screened, and its production described.

ET 05 Small group teaching for classes of 50+ and an element of peer assessment

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An exercise that develops skills in information retrieval, abstracting, written and spoken communication, discussion and decision-making

forms part of a Term 1 microbiology module. In Week 1, a member of the library staff gives a talk on the use of the library and the internet for information retrieval using an on-line demonstration of access to the library help page. Students are then required to research a topic allocated from a set of 8 topics with the help of this guidance and prepare short notes including references for submission in Week 2 for assessment.

Group discussions take place in Week 2 at which members share their findings and collectively prepare a short talk to be given to the whole class by a spokesperson. Thus each student learns about one topic in some detail and gains an overview of the other 7.

The session in Week 3 includes a peer review exercise in which each group reviews, makes written comments on and places in rank order a set of anonymous notes of a different group. The peer reviews are moderated by the class supervisor and graded.

The exercise is repeated in Weeks 4 and 5 with a different set of topics.

ET 06 Integrating training in library skills into first year undergraduate microbiology teaching

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A well-established study skills exercise forms part of a Term 1 microbiology module. Each student draws on readily accessible sources of information to prepare a short biographical note on the work of a famous microbiologist. Although general advice is given during optional tours of the library, in the past no specific guidance on information retrieval had been included in the exercise. Therefore, the value of integrating professional training into the exercise was investigated through a collaborative project between a member of the university library and the teacher.

For the investigation, a member of university library staff took part in an introductory session. The class of some 150 students was divided into two groups; the control group (starting in week 1) received no help, as hitherto; the test group (starting in week 6) was given guidance about access to general help and information specific to the exercise available from the library. The abilities and experiences of both groups were obtained by questionnaire before and after the exercise. The results suggested that the test group had developed enhanced information handling skills and confidence in sourcing information. Few of the control group sought help from library staff.

Posters

Fermentation & Bioprocessing Group

FB 01 The purification of the recombinant vaccinia virus B5R protein from a baculovirus expression system

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The causative agent of smallpox is *variola* virus. The current smallpox vaccine is comprised of live *vaccinia* virus, which is applied to the skin by scarification resulting in a localised infection. Inflammation and virus shedding occur at the resulting lesion, which represents a source of secondary transmission. Moreover, young children and immuno-compromised vaccinees are highly susceptible to complications from the live vaccine. Therefore, an alternative vaccine preparation based on components of the existing smallpox vaccine is sought.

Initial studies highlighted three *vaccinia* virus genes, that when administered to mice as DNA vaccines offered measurable levels of protection against an intranasal challenge with a lethal dose of *vaccinia* virus strain IHD. B5R was one of these virus genes, which was also cloned to produce a recombinant sub-unit vaccine, to promote enhanced humoral immunity. Expression of a B5R gene fragment in *E. coli* was good but an alternative, eukaryotic expression system was adopted to produce this glycoprotein. A recombinant baculovirus was constructed to secrete recombinant His-tagged B5R protein in cultured insect cells.

Preliminary purification methods utilised large volumes of tissue culture supernatant, which was manually applied to a 1 ml His-trap affinity column (Amersham Biosciences). This method recovered very small yields of the B5R protein as detected by Western blotting. Therefore, to reduce the large volumes involved, an ammonium sulphate precipitation step was introduced in the purification process. This step successfully concentrated the protein prior to affinity purification on an Akta FPLC automated purification system (Amersham Biosciences). Eluted fractions were analysed by SDS-PAGE and Western blot analysis, before assaying for protein content via BCA protein assay. Sufficient purified B5R was produced for use in animal studies.

FB 02 Sporulation and δ-endotoxins synthesis during fed-batch culture of *Bacillus thuringiensis* serotype H14

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Bacillus thuringiensis produces parasporal bodies during sporulation that contain proteins named δ-endotoxins which are toxic to insect larvae upon ingestion. *Bacillus thuringiensis* H14 is toxic to certain nematocerous dipterans, particularly mosquitoes and blackflies. Its parasporal body is basically spherical/ovoidal that contains at least three major proteins of 25, 65 and 130 kDa. Synthesis of these proteins was studied during fed-batch culture and sporulation of this bacterium using sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). The culture was continued until 48 h where the sporulation was 93%. The first synthesised protein was the 65 kDa protein. It was appeared at 16 h of culture where the sporulation was still poor. The 25 and 130 kDa were appeared at 18 h and 23 h correspondingly where the sporulation was less than 20%. The 25 and 65 kDa proteins were enriched in the course of fermentation but the 130 kDa protein had not the same progressive enrichment.

FB 03 Efficient production of human salivary cystatins by *Bacillus subtilis*

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Cystatins are physiological cysteine protease inhibitors serving a regulating function to prevent inappropriate proteolysis, which could be harmful or lethal. The availability of large amounts of human cystatins promises to provide a hint for effective utilization of them. Based on the idea, we attempted to develop a *Bacillus subtilis* system enabling the large-scale production of engineered human salivary cystatins (S, SA, and SN) that exceeds an *E. coli* system in the efficiency of production. The DNA fragments coding for mature cystatins (121 residues for each cystatin) were ligated in frame to the DNA segment for the signal peptide of endoglucanase in the pHSP-US plasmid vector. The *B. subtilis* lacking the alkaline protease E gene was transformed with the expression vectors. The transformant carrying the expression vectors was cultivated in a jar-fermenter for 3 days at 30°C. After fermentation, the culture medium was collected by centrifugation. Recombinant cystatins were purified by ion-exchange chromatography from the medium. The yield of each inhibitor was elucidated to be one gram for 1-litre cultivation. The recombinant cystatins were considered to be equivalent to native inhibitors. In conclusion, we have developed an excellent B. subtilis system supplying the human cystatins of family 2.

FB 04 Purification of *F. tularensis* vaccine candidates expressed in the RTS cell free expression system

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Francisella tularensis is the causative agent of tularemia a debilitating disease of man and animals. It exists as two biotypes, type B and the more virulent type A. The current vaccine against Tularemia is based on the live vaccine strain (LVS) which is an attenuated Type B strain and is not fully licensed for human use. The aim of our research is to identify potential sub-unit antigens that may be developed as a tularemia sub-unit vaccine. Fifty proteins have been selected for assessment, twenty five by the use of an algorithm to predict antigenic proteins and 25 by MHC epitope prediction. The genes encoding the selected proteins were cloned into the expression vector pCR®T7 TOPO® which allows expression with a poly-6-His tag. Proteins were expressed in the Roche RTS 500 and proteomaster cell free expression systems prior to purification by IMAC.

FB 05 The penicillin binding proteins of *Streptomyces coelicolor*

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We are attempting to knock out the high molecular weight penicillin binding proteins (PBPs) of *Streptomyces coelicolor*, using the Redirect method and transposon mutagenesis. Currently we have deleted the following genes, SCO 2090, SCO2608, SCO2897, SCO3580, SCO3771, SCO3847, SCO3901 and SCO5039. The resulting mutants are being characterised at a physiological level.

Of the mutants characterised none have been found to be lethal, suggesting that, as in other organisms, there is redundancy amongst the PBP genes. Phenotypically the most overt mutant is that of SCO2608 (pbp2 homologue), which appears to be linked to the arrangement of 'normal' spore formation. Currently we are examining the relationship between mutants and their ability to sporulate, branch and septate.

In the course of this work we have established a link between the exposure of *S. coelicolor* in continuous culture to penicillin and the development of resistance to the antibiotic. The resistance appears in part to be the consequence of a low molecular weight PBP that is loosly associated with the cell wall. We have attempted to isolate this protein but due to its low abundance we have not been able to produce sufficient protein to facilitate sequence analysis.

FB 06 Alteration of *Saccharomyces cerevisiae* (Baker's yeast) amino acid profiles by manipulation of fed-batch and continuous fermentation processes

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Saccharomyces cerevisiae (Baker's yeast) is one of the most important organisms within the industrial world. For centuries, it has had a long record of application within the production of food and alcoholic beverages, given it the classification as a GRAS organism (generally regarded as safe).

The use of fed-batch is a well-established fermentation for the production of a high-cell density, and it is generally this method that produces the biomass of baker's yeast. These cells are a good source of protein and Baker's yeast represents the largest bulk production of any single-cell micro-organism in the world.

The purpose of this work was to develop a high cell density fed-batch Baker's yeast fermentation and then alter certain parameters within this bioprocess. By doing so this work not only looked at the key factors affecting yeast growth, but also at specific changes within the Baker's yeast amino acid concentrations.

Using continuous culture and defined synthetic media, certain aspects of the chemical ratios were altered to show changes on the amino acid profiles.

The influence of certain operative conditions within the fermenter had an impact on final biomass and the Baker's yeast amino acid profile also showed variations throughout the fermentation process.

FB 07 Determination of media composition on morphology and capreomycin biosynthesis by *Streptomyces capreolus*

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Tuberculosis has re-emerged as one of the leading causes of death. The estimated 8.89 million new cases every year correspond to 52,000 deaths per week or more than 7,000 each day.

Capreomycin, produced by *Streptomyces capreolus* is used as a secondary antituberculous drug to treat patients with multi-resistant *tubercle bacilli*, and is active against streptomycin and viomycin resistant organisms. It comprises four species, capreomycin IA and IB, the active forms of the antibiotic and minor products capreomycin IIA and IIB.

Limited research has been carried out on both *S. capreolus* and capreomycin since its discovery in 1962, and so in conjunction with Eli Lilly and Company Limited, research is being undertaken to gain a better understanding of capreomycin biosynthesis. A comparison has been made between a minimal medium, largely composed of minerals and a limited carbon and nitrogen source, and a complex medium currently utilised by Eli Lilly, comprising complex carbon and nitrogen sources, and selected minerals.

Shakeflasks were used to culture *S. capreolus* at 30°C over 168 h. Mycelial pellets with distinct structural differences were observed in both media, whilst fragmentation into rod-like cells occurred readily at 48h in the minimal medium and was absent from the complex medium. Fluorescence microscopy was used to determine morphological differences, whilst reversed-phase HPLC quantified capreomycin production. Results suggest that the 'stressed' environment induced by pelleting does not contribute solely to antibiotic production and fragmentation is deleterious to capreomycin biosynthesis.

The nitrogen source in the complex medium plays a vital role in capreomycin biosynthesis. This may be linked to the inclusion of certain amino acids in the antibiotic structure. Serine and alanine are directly incorporated into capreomycin IA and IB which also contain ß-lysine residues, whilst serine alone is indirectly incorporated into the IIA and IIB moieties. However, simply supplementing these amino acids does not contribute to higher yields. We therefore aim to determine the preferential utilisation of amino acids by *S. capreolus* and use this information to substitute the nitrogen sources in the current complex medium. Linking this with pellet formation will enable a chemically defined medium to be potentially optimised, reducing capreomycin yield variability, a problem with the current process. Reversed-phase HPLC and a pre-column derivatization technique will quantify labelled amino acids in the medium.

FB 08 Production of a recombinant protein in the methylotrophic yeast *Pichia pastoris* is affected by physical parameters within the bioreactor

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The methylotrophic yeast *Pichia pastoris* is an ideal host for the production of foreign gene products largely due to the efficient methanol induction system (Egli *et al.*, 1980) and the ability to grow to high cell density in fermenter cultures (Veenhuis *et al.*, 1983). *Pichia* is capable of a high degree of post-translational modifications, making it more suitable for the production of eukaryotic recombinant proteins, e.g. disulphide bond formation, correct folding, glycosylation, etc. (Cregg *et al.*, 2000). From an industrial perspective, *Pichia* is also an ideal system for the production of important heterologous proteins largely because the simple purification of secreted recombinant proteins is enabled due to low levels of native secreted proteins (Cregg *et al.*, 1993).

For these reasons, the *Pichia* expression system is often selected to produce functionally active proteins, especially those that have not been expressed efficiently in other microbial systems (Cereghino *et al.*, 2001b).

In this investigation, we have studied the effects of certain physical parameters within the bioreactor on the production of a heterologous

protein, e.g. temperature and pH. These parameters are known to play an important role in obtaining uncleaved and fully functional heterologous proteins and we demonstrate here the optimal parameters to achieve this with our particular gene product.

FB 09 Monitoring of a continuous submerged fungal bioprocess using near infrared spectroscopy

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Bioprocess monitoring and control could be significantly improved through the introduction of a technique capable of measuring chemical and biological parameters in a fermentation process simultaneously and in real time. Near infrared spectroscopy, (NIRS), is a non-destructive, rapid and versatile technique that can be used to monitor the concentrations of several key analytes in a complex bioprocess contemporaneously.

The initial aim of this work was to use NIRS to develop at-line models for key analytes, including biomass, glucose and ethanol, and subsequently transfer these principles to *in-situ* modeling using a fibre optic probe. Much of the published work has used batch or fed-batch bioprocesses, where there is a visible change in the concentrations of key analytes within the sample matrix. A continuous fungal bioprocess presents additional challenges due to the filamentous nature of the micro-organism, and because little to no variation in the concentration of analytes in the sample matrix exists.

A range of different approaches to the modeling process have been investigated in order to optimise the robustness and precision of the models developed. This work has demonstrated the potential of NIRS for monitoring a complex bioprocess whereby there is minimal variation in analyte concentrations during the steady state.

FB 10 Microbial mucinolytic enzymes – to aid the treatment of respiratory CF patients

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Cystic fibrosis patients, as a consequence of the genetic disorder in the transmembrane regulator (CFTR), have dehydrated airway surfaces, hypersecretion of viscous mucin, coupled with ineffective mucociliary clearance. This leads to increased susceptibility to opportunistic bacterial pathogens, including, amongst others, *Pseudomonas aeruginosa, Burkholderia cepacia, Staphylococcus aureus*, *Haemophilus influenzae*.

The overall aims of the PhD studentship are to identify and isolate novel microbial mucinolytic enzymes to aid the treatment of respiratory cystic fibrosis patients. A novel drug delivery package is aimed to be designed that should achieve effective incorporation and release of the enzyme and relevant respiratory drug.

Robust, sensitive and effective screens have been developed as tools for efficient detection of potential mucin-degrading microbial enzymes or enzyme cocktails. Two levels of monitoring and evaluation of enzyme activity have been used. The spectrophotometric assay involved detecting a decrease in turbidity of mucin in the presence of the microbial mucin-degrading enzyme(s), whereas the plate assay displayed zones of clearing around the cultures that produced these enzymes.

Due to the high molecular weight and extended size of the mucous glycoproteins, their characterisation has proven somewhat difficult. Electrophoresis through combined Agarose-Polyacrylamide gels has permitted visualisation of mucins and the breakdown products of these glycoproteins by crude mucinolytic enzyme preparations.

Posters

Microbial Infection Group

MI 01 The role of HtrA in the virulence and competence of *Streptococcus pneumoniae*

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HtrA (High Temperature Requirement A) is a heat-shock protein that acts as a molecular chaperone at low temperatures and as a protease at high temperatures. It prevents the accumulation of denatured or misfolded proteins resulting from stressful conditions such as temperature increase or oxidative stress. In the present study, we used our pneumonia and systemic models of infection to investigate the role of HtrA in the virulence of *Streptococcus pneumoniae* strains, D39 (type2) and TIGR4 (type4). Deletion of the gene for HtrA from strain D39 completely abolished virulence in both mouse models while that of TIGR4 was dramatically reduced. HtrA-negative mutants induced much less inflammation in the lung during pneumonia. HtrA was also involved in the ability of the pneumococcus to grow at high temperature, to resist oxidative stress and to undergo genetic transformation. Reduced virulence and competence of D39Δ*htrA* mutant could be restored by expression of HtrA. Our results demonstrate that HtrA is a key virulence factor of *S.pneumoniae*.

MI 02 An *in vitro* assay to investigate the ability of CpG-DNA to stimulate macrophage cells to control *F. tularensis* infection

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Stimulation of innate immunity by CpG-DNA has been shown to protect against a number of intracellular pathogens. Elkins *et al* demonstrated that a single dose of CpG-DNA administered 3 days before challenge offers protection to 10^3 cfu *F. tularensis* LVS i.p. in the BALB/c mouse model. However, whilst LVS is virulent in mice it is attenuated in man. We have developed an *in vitro* assay to study the ability of J774 murine macrophages stimulated with CpG-DNA to control *F. tularensis* infection. We intend to use this assay to screen for CpGs which may be effective in the animal model of disease. J774 cells were stimulated with CpG 1826 and infected with LVS. Extracellular bacteria were removed by washing and gentamicin treatment and the cells cultured for a further 48 hours in the presence of CpG-DNA. At various time-points cells were lysed and intracellular bacterial numbers determined by plate counting. CpG 1826 stimulated cells were able to control the intracellular replication of LVS when compared to control cells. This work will continue by investigating the ability of CpG 1826 to stimulate J774s to control the intracellular replication of the fully virulent strain of *F. tularensis* HN63.

MI 03 Characterisation and identification of a meningococcal T-cell stimulating protein (TspB)

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Introduction: TspB (T-cell stimulating protein B) has been identified as a highly immunogenic meningococal-specific protein that may be surface exposed and provide a good target for bactericidal antibodies. TspB was isolated during the screening of a λZapII genomic library with rabbit serum raised against an extract of *N.meningitidis* enriched for $CD4^+$ T-cell stimulating outer membrane antigens.

Results and conclusion: TspB was bioinformatically analysed and it is present in the genome of the sequenced serogroups A, B and C strains. The gene is within a horizontally transferred pathogenicity island. The *tspB* gene has been cloned in an *E.coli* expression vector and the protein has been purified. The T-cell stimulating capability of the protein was confirmed, using $CD4^+$ T-cell proliferation assays. Rabbit polyclonal antibodies were raised against the denatured purified protein in a New Zealand White female rabbit. Using immunoblots, the antiserum detected the presence of TspB in different meningococcal clinical isolates. Furthermore a defined *N. meningitidis* TspB mutant has been constructed in which the gene has been disrupted by insertion of an antibiotic cassette. The sub cellular localisation of TspB and the bactericidal activity of its antibodies will be investigated. Also, detailed molecular, immunochemical and biological properties of TspB will be carried out.

MI 04 The plasmid encoded FinB invertase of *Bacteroides fragilis* is present in isolates of both clinical and faecal origin

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The genome of *Bacteroides fragilis* contains a number of invertible promoter regions that control, for example, polysaccharide biosynthesis and are related to the antigenic variation demonstrable by monoclonal antibody labelling and immunofluorescence microscopy. The inversion of these DNA regions is thought to be mediated by a mechanism similar to the Hin-system which controls the expression of two antigenically different flagella in *Salmonella*. The DNA inversion is mediated via the association of the Hin invertase enzyme with 30–32bp regions of dyad symmetry that bound the invertible regions of DNA. The *B. fragilis* NCTC9343 genome sequencing project has revealed two Salmonella *hin* homologues, *finA*, located on the chromosome and *finB* located on a 36.5 kb plasmid. The aim of the current study was to determine whether *finA* and *finB* are found similarly in a range of other strains of both faecal and clinical origin. The results indicate that the plasmid carrying *finB* is present in a minority of strains of both faecal and clinical origin.

MI 05 Effect of polysaccharide expression on the antimicrobial susceptibility of *Bacteroides fragilis*

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A number of factors may contribute to the virulence and antimicrobial resistance of *Bacteroides fragilis* with the production of extracellular polysaccharides considered to play a key role. The aim of this study was to determine the effect of polysaccharide

capsular expression on the antimicrobial susceptibility of *B. fragilis* by examining the susceptibility of large capsule (LC), small capsule (SC) and electron dense layer (EDL) sub-populations of *B. fragilis* strain NCTC 9343 to a selection of antibiotics. The minimum inhibitory concentrations (MICs) of cefoxitin, piperacillin/tazobactam, co-amoxiclav, metronidazole, meropenem, clindamycin and ciprofloxacin for non-enriched and subpopulations of NCTC 9343 enriched for LC, SC and EDL were determined in Brucella broth using the broth microdilution method as recommended by the National Committee for Clinical Laboratory Science. Differences in polysaccharide expression did not appear to have an effect on antibiotic resistance with all MIC values for a given antibiotic being similar to within one dilution for enriched subpopulations. The results of this study suggest, therefore, that differences in polysaccharide expression in Brucella broth do not affect the MIC of *B. fragilis*.

MI 06 The human-specificity of intermedilysin is determined by the last 56 residues of domain 4

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Intermedilysin (ILY) is a pore-forming toxin belonging to the gene family of the cholesterol dependent cytolysins (CDCs). It is unique within the group in that it exhibits human-specific cytolysis and it has been suggested that this is due to binding of a different cellular receptor to other toxins of the family.

In order to identify the region responsible for the human specificity of intermedilysin, we created a bank of chimeras between intermedilysin and pneumolysin. The chimeric toxins were expressed, purified by immobilised metal affinity chromatography, and the specificity of the mutants was determined by haemolytic assay on human and rabbit erythrocytes. It was found that the specificity of the chimeric toxin was determined by the origin of the C-terminal 53/56 residues, indicating that this region of domain 4 is responsible for the human specificity of intermedilysin.

MI 07 *Acanthamoeba* induces host cell death via a phosphatidylinositol 3-kinase (PI3K)-dependent mechanism

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Acanthamoeba is an opportunistic protozoan parasite, which can cause fatal granulomatous amoebic encephalitis (GAE) and eye keratitis. However, the pathogenesis and pathophysiology of these emerging diseases remains incompletely understood. Aims of this study were to determine (i) whether *Acanthamoeba* induces host cell signalling pathways and (ii) the role of PI3K in *Acanthamoeba*-mediated host-cell death.

Two isolates of *Acanthamoeba* belonging to T1 genotype (GAE isolate) and T4 genotype (Keratitis isolate) were used and their pathogenic potential determined using human brain microvascular endothelial cells (HBMEC) and human corneal epithelial cells (HCEC), respectively. To determine host cell signalling pathways in response to *Acanthamoeba*, tyrosine phosphorylation of HBMEC proteins were examined following stimulation with *Acanthamoeba* using antiphosphotyrosine antibody, 4G10 in Western blotting assays. To determine whether PI3K plays a role in *Acanthamoeba*-mediated host cell death, cytotoxicity assays were performed using both PI3K dominant negative host cells and PI3K inhibitor, LY294002. PI3K activation in response to *Acanthamoeba* was determined using antibodies against its downstream substrate, Akt.

Both *Acanthamoeba* isolates produced severe cytotoxicity (up to 70%) on HBMEC and HCEC as determined by LDH release assays. Additionally, *Acanthamoeba* induced host-cell apoptosis within 3 h, indicating the modulation of host-cell signalling pathways. Western blotting studies revealed that *Acanthamoeba* induced tyrosine phosphorylations on several HBMEC proteins (approximate Mw120, 80, 68, 48, 44, 36, 30 kDa). Furthermore, Akt was shown to be directly activated in response to *Acanthamoeba* suggesting the role of PI3K in *Acanthamoeba*-mediated host cell death. To this end, more than 65% host cell death was inhibited using PI3K inhibitor, LY294002. These data were further confirmed using host cells expressing dominant negative forms of PI3K.

Taken together, we have shown that *Acanthamoeba* induces host cell signalling pathways and identified PI3K as a crucial molecule that mediates host cell death due to *Acanthamoeba*. A complete understanding of *Acanthamoeba*-host cell interactions may help develop novel strategies to prevent these serious infections.

MI 08 Potential virulence profile of *Aeromonas* spp., *Vibrio* spp., and *Plesiomonas shigelloides* isolated from different sources in the Philippines and Thailand

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Aeromonas spp., *Vibrio* spp., and *Plesiomonas shigelloides* belong to the expanding group of known water-borne pathogens. Thirty-nine (39) isolates comprising of several species of *Aeromonas* (16), *Vibrio* (21) and *P. shigelloides* (2) were isolated from aquatic environments, aquatic animals and human diarrhoeal patients in the Philippines and Thailand. The potential virulence profile of these bacteria was determined and compared. Production of cytotoxin was investigated using Vero cell monolayer. Cell associated and cell free β-haemolysin were examined in 5% Blood Agar Plates (BAP). Adhesion to human intestinal (Henle 407) cells was compared among *Aeromonas* spp. isolated from clinical and environmental sources. The occurrence of haemolysin genes: tlh, tdh; and urease activity was investigated among *V. parahaemolyticus* isolates. Results revealed that isolates from humans and fishes produced both cytotoxin and β-haemolysin. Except for one *V. cholerae* Non 01, all the isolates from the environment were non cytotoxic and non haemolytic. Only those *Aeromonas* isolates from clinical sources adhered to Henle 407 cells. All *V. parahaemolyticus* isolates, which were recovered from the environment were tlh+, tdh– and urease–. It is apparent that the potential virulence profiles investigated in the present study were different from clinical sources as compared to those of environmental sources. It is probable that these potential virulence factors could be involved in the pathogenesis of the disease in both humans and animals.

MI 09 Sequencing of *ply* genes from clinical pneumococcal isolates shows high levels of conservation

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Pneumolysin (Ply) is an important virulence factor of *Streptococcus pneumoniae*, and is a member of the family of pore forming toxins known as Cholesterol-Dependent Cytolysins (CDCs). The *ply* gene is present in all pneumococci and believed to be well conserved. Ply is required for full invasive disease and has a range of detrimental effects on the host during infection. Pneumolysin toxoids are being considered as vaccine candidates.

Amplification and sequencing of *ply* from 250 clinical isolates of *S. pneumoniae* was carried out using liquid handling robotics and a high throughput 96 capillary DNA sequencer (MegaBACE, Amersham). Analysis of *ply* sequence data confirmed that the gene is highly conserved with the majority of variation the result of single nucleotide polymorphisms. We observed the presence of Ply proteins containing a two amino acid deletion in certain serotype 7 and 8 pneumococci as previously reported. We also discovered this mutation in a serogroup 23 isolate. In addition we have identified a serotype 1 pneumococcus containing a novel 8-amino acid insertion.

This work was funded by the Chief Scientist Office of the Scottish Executive.

MI 10 The structure and function of meningococcal T-cell stimulating protein A (TspA)

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Introduction: TspA is a high molecular weight, T-cell and B-cell stimulating protein of *Neisseria meningitidis*. TspA has a highly positively charged N-terminus, which contains a putative peptidoglycan-binding domain. There is a hydrophobic putative trans-membrane region and a highly negatively charged C-terminus containing a multiple repeat region and a coiled coil. In this study the molecular features and function of TspA were investigated.

Results and Discussion: An isogenic deletion mutant and a bank of truncated *tspA* mutants were produced. Homology to fimV, the *Pseudomonas* gene involved in twitching motility, suggested a link between TspA and the type IV pilus. Since pili are important in the association of meningococci to human cells, the adherence of TspA mutant bacteria and their wild type counterparts were compared. The mutants were reduced in their ability to adhere to cell culture monolayers. However, twitching motility stab assays showed no differences between wild type and mutants.

Conclusion: These findings imply that TspA interacts with the meningococcal pilus. Further work on transformation competence and electron microscopy is underway to establish this link.

MI 11 The *Pasteurella multocida* toxin is encoded within a lysogenic bacteriophage

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Toxigenic strains of *Pasteurella multocida* produce a 146 kDa toxin (PMT) that acts as a potent mitogen. Sequence analysis of the structural gene for PMT, *toxA*, previously suggested it was horizontally acquired, since it had a low G+C content relative to the *P. multocida* genome. To address this, the sequence of DNA flanking *toxA* was determined. The sequence analysis showed the presence of homologues to bacteriophage tail protein genes and a bacteriophage antirepressor, suggesting that the toxin gene resides within a prophage. In addition to phage genes, the *toxA* flanking DNA contained a homologue of a restriction/modification system that was shown to be functional. The presence of a bacteriophage was demonstrated in spent medium from toxigenic *P. multocida* isolates. Its production was increased by mitomycin C addition, a treatment that is known to induce the lytic cycle of many temperate bacteriophages. The genomes of bacteriophages from three different toxigenic *P. multocida* strains had similar but not identical restriction profiles, and were approximately 40–50 kb in length. The prophages from two of these had integrated at the same site in the chromosome, in a tRNA gene. Southern blot analysis confirmed that these bacteriophages contained the *toxA* gene.

MI 12 Investigation of pneumococcal sortase

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Sortase enzymes, found throughout Gram-positive bacteria, are responsible for the covalent anchoring of specific proteins to the cell wall. The contribution to bacterial pathogenesis of these cell wall anchored proteins mean that sortase are important virulence factors in many Gram-positive pathogens and are seen as a potential target for novel antimicrobials.

Streptococcus pneumoniae (the pneumococcus) is an important human pathogen causing pneumonia, bacteraemia, otitis media and meningitis. The sequenced genome from two

S. pneumoniae strains revealed four sortase genes, *srtA-D*. The unequal strain distribution of these genes in the sequenced genomes promoted an examination of their distribution among a large collection of clinical isolates. *srtA*, unlike the other three sortase genes, was found to be ubiquitous among all strains and so was investigated further in terms of its role in mouse infection and colonisation and localisation of cell wall anchored proteins.

MI 13 Interaction of probiotic lactobacilli and pathogens in a Hep-2 cell adherence assay

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The mechanisms by which probiotics can have a beneficial effect on human health are still unclear. There is some evidence lactobacilli can help protect against infection by pathogens, this could be by direct exclusion at epithelial surfaces and / or production of antibacterial substances. Inhibitory activity of 26 probiotic lactobacill strains (9 species) against the pathogens enteroaggregative *E. coli* and *Ps. aeruginosa* was investigated using an epithelial cell adherence model. This involved co-incubation of lactobacilli and pathogen on Hep-2 cell monolayers, then assessment of adherence relative to that seen with mono-incubation. An agar growth inhibition assay was also used to see if the lactobacilli could directly inhibit growth of the pathogens.

All the lactobacilli adhered well to Hep-2 cells, as did the pathogens. When co-cultivated, there was no evidence of pathogen cells being inhibited from adhering to the Hep-2 cells, i.e. the presence of a probiotic did not alter the level of pathogen adhesion to the cultured cells. Apart from *L. delbruckei*, all the lactobacilli inhibited growth of the pathogens in the agar assay. For these probiotics, direct inhibition of pathogen growth may be of more importance in protecting the host than preventing adherence of the pathogens to epithelial surfaces.

MI 14 Bactericidal activity of phagocytes changed in respect on *Staphylococcus aureus* and *Listeria monocytogenes*

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The gram-positive bacteria *Staphylococcus aureus* and *Listeria monocytogenes* were known to differ by mechanism of intercellular multiplying in phagocytes. By contrast from *S. aureus*, which are activity ingested within phagosoms, *L. monocytogenes* is able to penetrate in the interior of phagocytes and develops an intracellular life cycle. In this study, we tested level various functions of guinea pig's blood and foci inflammation phagocytes (monocytes and neutrophils) infected by virulence and avirulence strains *S. aureus* and *L. monocytogenes*. We investigated functional (formation of NO – reaction with Griess reagent, of free radical activity oxygen – NBT-test), phagocytic and cytochemical (ATF-asa, 5'-nucleotidase, myeloperoxidase) activity of phagocytes.

The results demonstrated that activity of phagocytes of infection animals depends from type of the agents. So, in *L. monocytogenes* infection the blood cell were more actively stimulated, than phagocytes of animals infected by *S. aureus*. The increasing activity of nitric oxides (NO) and oxygen-depend bactericidal systems of phagocytes in different periods of infection indicated on this. This activity increased significantly ($P<0.01$) from data of intact animals after 5 hours *L. monocytogenes* infection, then in *S. aureus* infection after 7 h. In turn, the NO-depend bactericidal system of phagocytes was more activated in *L. monocytogenes* infection.

The stimulation of foci inflammation phagocytes of guinea pig's infected with virulent strain *S. aureus* was considerably enhanced at more early periods (5 h). The data of activity extoenzymes ATF-asa and 5'-nucleotidase showed this. On the contrary, this activity of cells of animals infected with avirulent strain *S. aureus* revised significantly ($P<0.01$) in the later period (7 h). Discovered, that blood phagocytes of animals infected with avirulence strain *L. monocytogenes* were stimulated significantly ($P<0.01$), than of foci inflammation cells. While virulence strain activated NO-depend bactericidal system of phagocytes more strongly, then avirulence strain.

Thus, we revealed that a functional activity of neutrophils and monocytes/macrophages depends from type and degree virulence of gram-positive bacteria.

MI 15 Interspecies transfer of antibiotic resistance genes in bacteria

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The increasing occurrence of antibiotic resistance amongst bacterial species is a serious problem. This prevalence of antibiotic resistance is associated with horizontal transfer of genes between bacteria. Of the several methods by which horizontal gene transfer may occur conjugation is thought to be the most common.

The potential for antibiotic resistance genes to transfer between pathogenic and spoilage bacteria in the food chain, and thence to the gastro-intestinal tract microbiota, has led to renewed interest in interspecies and intergeneric gene transfer between bacteria. Although conjugal transfer has been implicated in this phenomenon there is limited detailed information on the mechanisms involved. This project investigates how environmental conditions found in the food chain and the gastro-intestinal tract might affect the transfer of conjugative genetic elements between a range of Gram-positive and Gram-negative bacteria.

MI 16 The role of prophage-like elements in the diversity of *Salmonella enterica* serovars

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The *Salmonella enterica* serovar Typhi CT18 (*S.* Typhi) chromosome harbours seven distinct prophage-like elements, some of which may encode functional bacteriophages. In silico analyses were used to investigate these regions in *S.* Typhi CT18, and ultimately compare these integrated bacteriophages against 40 other *Salmonella* isolates using DNA microarray technology. *S.* Typhi CT18 contains prophages that show similarity to the lambdoid, Mu, P2 and P4 bacteriophage families. When compared to other *S.* Typhi isolates, these elements were generally conserved, supporting a clonal origin of this serovar. However, distinct variation was detected within a broad range of *Salmonella* serovars; many of the prophage regions are predicted to be specific to *S.* Typhi. Some of the P2 family prophage analysed have the potential to carry non-essential 'cargo' genes within the hyper-variable tail region, an observation that suggests that these bacteriophage may permit a level of specialisation on its host. Lysogenic bacteriophages therefore play a crucial role in the generation of genetic diversity within *S. enterica*.

MI 17 Two distinct binding sites on the tetanus toxin H_C fragment are essential for ganglioside binding

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Tetanus toxin (TeNT) from *Clostridium tetani* consists of an N-terminal enzymatic L chain (50 kDa) disulphide bonded to a C-terminal (100 kDa) H chain, responsible for binding, trafficking and cytosolic escape of the L chain. H_C, the 50 kDa C-terminal fragment of the H chain, retains the binding and trafficking characteristics of whole toxin. Structural data suggests there are two distinct ganglioside-binding sites on H_C; one binds Gal-GalNAc, the other binds sialic acid.

Mutant H_C proteins containing amino acid substitutions within both sites were constructed and purified from *E. coli*. Proteins were assayed for binding to GT1b gangliosides and to N18 RE-105 neuroblastoma cells.

Our results confirm that both sites on H_C are essential for binding ganglioside. GT1b binding of Gal-GalNAc site mutants is reduced to below 13% of wild type and binding of one mutant in the sialic acid is reduced to 10%, though it still binds cells. Surface plasmon resonance analysis indicates that H_C does not bind ganglioside in a 1:1 stoichiometry and native PAGE suggests H_C migrates as a tetramer. Pre-incubation of mutant proteins with GT1b and analysis by native PAGE demonstrated retardation of mutants with near wild type ganglioside binding, but not of mutant proteins.

MI 18 Mucosal adjuvant activity of a 14 repeat sequence from Toxin A from *Clostridium difficile*

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Toxin A is one of the major virulence determinants of *Clostridium difficile*. A striking feature of this toxin is the repetitive nature of the amino acid sequence at the C-terminus, which encodes the receptor-binding domain. Recombinant His-tagged derivatives of the toxin, which possess different numbers of these repeats, have been cloned, expressed and purified from *Escherichia coli*. One of these proteins (14CDTA-His) demonstrated mucosal adjuvant activity in mice, enhancing local and systemic immune responses to co-administered, nontoxic C-terminal domain from tetanus toxin known as fragment C. We have further evaluated the systemic and mucosal immunogenicity of this protein in mice. In addition the cellular responses to both the toxin and a normally non-responder bystander antigen ovalbumin have been established.

MI 19 Comparison of novel conjugates derived from botulinum neurotoxins

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Work at HPA Porton Down over the last few years has established a capability to produce conjugates consisting of fragments of botulinum neurotoxins that retain endopeptidase activity but with the cell binding activity of the native neurotoxins replaced by alternative cell targeting ligands. This technology has been used to develop molecules based upon a variety of botulinum neurotoxin serotypes with different SNARE protein substrate specificities, and employing a range of targeting ligands. We have compared the properties of the ligand targeted conjugates and native neurotoxins in a variety of *in vitro* cellular systems. The results of these comparisons provide insights into the role of the different domains of the toxin molecule in the overall properties of the botulinum neurotoxins, and also into the basis of some of the functional differences between serotypes, particularly in relation to duration of action.

Posters

Physiology, Biochemistry & Molecular Genetics Group

PBMG 01 Light emission from bacteria in condition of their optical interaction

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There is increasing evidence for the light-mediated communication in bacterial cultures. Previous studies shown that cell division, adaptive and adhesive capabilities of bacterial cells might be connected with transmission of light signals that are forms within the cells. The investigation of light emission from bacteria in condition of their optical interaction (i.e. without chemical exchange between them) was the aim of the present study.

Experiments were performed with *Escherichia coli* MC1061 cells, which were cultured in a special device (i.e. separated into equal compartments glass cylinder) with the use of LB and M9 nutrient media. Photo-emission from bacteria at different stages of growth were measured using the FEU-69 photomultiplier, wihich was sensitive in the 450–800 nm range.

It was found that during lag phase of growth M9-cultured cells had a lower level of emission over the range of 450 – 675 nm. During active growth, both M9 and LB-cultured cells showed more intensive light emission in comparison with control cells. At stationary phase of growth, there was reduction in light emission intensities but only in LB-cultured cells.

Thus, the findings above demonstrates that light emission alter due to culture-to-culture optical interaction.

PBMG 02 *Echo*base: a post-genomic database for gene/function prediction in *Escherichia coli*

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The completion of the *Escherichia coli* K-12 genome sequence revealed that our knowledge of the functions of *E. coli* gene products is a long way from being complete. Currently there are still around 1700 genes that are annotated as 'function unknown' (FUN), which are being characterized at a rate of around 30 per year. High-throughput post-genomic experiments, as well as traditional biochemical and genetic experiments, are generating large datasets that relate to many FUN genes and we have created a relational database that integrates information from these experiments into a single resource. The database, *Echo*base, will then help to provide predictions for functions of these uncharacterised FUN genes. We will demonstrate the power of integrating information relating to a particular FUN gene from divergent experiment types, including microarrays, proteomics and bioinformatic studies. *Echo*Base also includes an analysis of 'orphan' and 'missing' enzymes that have been identified or predicted for *E. coli* from biochemical studies and metabolic reconstructions and have not been linked to a gene.

PBMG 03 Insecticidal toxin complexes from *Photorhabdus*

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Photorhabdus is an insect pathogenic bacterium vectored by a nematode. The bacteria reside in the gut of the infective juvenile nematode and are released directly into the insect blood system following penetration of the insect by the nematode. The bacteria then replicate within the insect, killing the insect host and also acting as a food source for the multiplying nematodes. Subsequently a new generation of infective juveniles reacquire the bacteria and emerge to colonise new insect hosts. To complete this complex lifecycle the bacteria must kill the insect host, by producing insecticides. We describe the isolation of the *toxin complex* genes encoding high molecular weight 'Toxin complexes' or Tc toxins which destroy the insect midgut. We discuss the composition of toxin genes required to produce oral toxicity to insects and also their recent expression in insect resistant transgenic plants.

PBMG 04 Structure of *N*-acylamino acid racemase at 1.3 Å: insights into the flexible binding pocket and the catalytic mechanism

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N-Acylamino acid racemase (NAAAR) that catalyzes racemization of *N*-acylamino acid is valuable to produce enantiopure α-amino acids in couple with an aminoacylase. In this investigation, NAAAR from a radiation-resistant ancient bacterium, *Deinococcus radiodurans,* was cloned and overexpressed. Enzymatic analysis showed that the expressed protein had NAAAR activity toward various substrates and an optimal temperature of 60°C. K_m values were 24.8 and 12.3 mM for *N*-acetyl-D-methionine and *N*-acetyl-L-methionine, respectively. The crystal structure of NAAAR was solved using MAD method and refined to 1.3 Å (R=13.8%). It reveals a tightly packed octamer and a classical architecture of the enolase superfamily comprising a capping domain and a $(\beta/\alpha)_7\beta$ barrel domain. The Mg^{2+}-bound structure was also determined and showed limited conformational change. A flexible pocket containing Lys170-Asp195-Glu220-Asp245-Lys269 catalytic site is thus identified, which suggests its role in diverging into distinct enzymes during evolution.

PBMG 05 *Propionibacterium acnes* and the intervertebral disc

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The underlying cause of sciatica and associated back pain is currently unresolved. Possible inflammatory or autoimmune aetiologies have been suggested. Stirling *et al* (2001) presented evidence for a possible link between sciatica and bacterial infection by demonstrating the presence of bacteria in disc material from 19 out of 36 patients with severe sciatica using a broth enrichment technique.

In a prospective study, intervertebral disc material from 87 patients undergoing spinal surgery was analysed for the presence of bacteria. Bacterial culture and non culture techniques including immunofluorescence microscopy (IFM) using specific monoclonal antibodies were applied to intervertebral disc and associated material retrieved during surgery.

Bacteria were isolated from disc material of 20% (n=13) of patients suffering from sciatica and from 9% (n=2) of a control group including scoliosis, trauma, tumour and degenerate disc patients. Bacteria were also identified in wound washings and wound tissue of some patients. Disc colonisation rates did not increase IFM detection. The anaerobe *Propionibacterium acnes* was the predominant bacterium isolated in both case and control groups. Sciatica patients were colonised by either *P. acnes* type 1, type 2 or, in three individuals, both.

We are unable to demonstrate an association between sciatica and infection in Northern Ireland (p=0.335).

PBMG 06 Mutasynthesis in *Streptomyces* for production of novel calcium-dependent antibiotics

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The calcium-dependent antibiotic (CDA) is a lipopeptide synthesised non-ribosomally and produced by *Streptomyces coelicolor* A3(2). CDA contains several non-proteinogenic amino acid residues. Hydroxyphenylglycine (4-HPG) is one of the unusual amino acids in the structure of the CDA and vancomycin groups of antibiotics. For the members of the vancomycin group of antibiotics, the 4-HPG residue plays crucial roles in the structure and function of the final glycopeptide antibiotic.

To reveal the putative biosynthetic pathway of this amino acid in CDA, a standard 'double crossover replacement strategy' was used to delete 4-hydroxymandelic acid synthase (4-HMAS, encoded by *hpd*) from *S. coelicolor* MT1110 and 2377, using the delivery plasmid pZMH3. There was no CDA production in the disrupted strains. Plates containing a gradient of hydroxymandelic acid were used to restore CDA production in both *S. coelicolor* MT1110Δ*hpd* and 2377 Δ*hpd*. Exogenous supply of 4-hydroxyl phenylglyoxylate and 4-hydroxyphenylglycine re-established CDA production by the *hpd* mutant. Feeding analogs of these precursors to the mutant resulted in the directed biosynthesis of novel lipopeptides with modified arylglycine residues (mutasynthesis).

PBMG 07 Characterisation of a metal ion dependent repressor in *Streptococcus uberis*

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Streptococcus uberis is one of the most significant aetiological agents of bovine mastitis.

Failure to control *S. uberis* infection is largely due to a lack of knowledge of the mechanisms by which it causes disease and there is accordingly an urgent need to improve our understanding of the molecular basis of *S. uberis* pathogenesis.

Growth and survival in a host requires a number of adaptive responses on behalf of bacteria, and the acquisition of metal ions is an important response for pathogenesis, with ion-limitation often serving as a key cue that an organism has entered a host.

Ion dependent control of gene expression in bacteria is coordinated by a number of known regulators e.g. the DtxR (diphtheria toxin repressor) and Fur (ferric uptake regulator) proteins. Following an *in silico* search of the *S. uberis* genome (currently at finishing stage) a DtxR homologue has been identified, and a mutant isolated from a screen of a bank of random insertion mutants. The function of this homologue, including metal ion specificity is currently being characterised using a combination of molecular biology and proteomics.

PBMG 08 Regulation of indol-3-eacetic acid biosynthesis in *Azospirillum brasilense* Sp245 by tryptophan

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The observation that the phytohormone indole-3-acetic acid (IAA) only accumulates in substantial amounts in the culture medium of many bacterial strains in the presence of an exogenous tryptophan supplement, suggests a crucial role for this amino acid in IAA biosynthesis. Tryptophan is a known stimulatory precursor of IAA biosynthesis, but its role in the expression of the indole-3-pyruvate decarboxylase gene (*ipdC*), encoding a key enzyme in the IAA biosynthesis pathway has been discussed. We tested the induction potential of tryptophan on the *ipdC* gene in both minimal and rich medium, using a translational *ipdc-gusA* fusion *pFAJ64*. The measured β-glucuronidase (GUS) activity was used to monitor the *ipdC* gene expression. In minimal medium, there was no β-glucuronidase activity in the absence of tryptophan, while β-glucuronidase activity was observed when medium was supplemented with trpytophan, suggesting that the *ipdC* gene may be regulated by tryptophan. In enriched medium, β-glucuronidase activity was high and similar with or without tryptophan supplement, pointing to the importance of minimal medium as a background for gene expression assays. We are showing for the first time that tryptophan, as low as 5µg/ml, can stimulate IAA biosynthesis as well as induce the *ipdC* gene expression.

Keywords: indole-3-acetic acid, tryptophan, indole-3-pyruvate decarboxylase, regulation.

PBMG 09 The construction of a *gfp*:*xylE* tandem reporter plasmid for use in *Actinobacillus pleuropneumoniae* (APL)

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We have constructed a reporter plasmid (pMIDG300) for use in APL, the cause of porcine pleuropneumonia. pMIDG300 contains a chloramphenicol resistance cassette and the promoterless tandem reporter genes *gfp* and *xylE*, downstream from a multiple cloning site (MCS).

The promoter regions of APL strain 4074 *rpoE* and *sodC* genes were cloned into the MCS of pMIDG300. *rpoE* and *sodC* encode the stress response sigma factor E (RpoE) and Cu,Zn superoxide dismutase respectively. In medium containing 3% ethanol, there was a 5-fold and 2-fold increase in GFP expression of the promoters *rpoE* and *sodC* respectively, compared to rich broth alone. In other organisms RpoE is negatively regulated by the anti-sigma factor MclA (RseA). Compared to wild-type, there was a 2.5-fold increase in GFP expression from the *rpoE* promoter in a *mclA* knockout mutant.

We conclude that pMIDG300 can be used to identify differential gene expression as a result of (1) changes in growth environment and (2) mutations in transcriptional repressors.

PBMG 10 Characterisation of the transient behaviour of *Synechocystis* sp. PCC6803 as an aid to metabolic pathways elucidation

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Synechocystis sp. PCC6803 is a useful model cyanobacterium for future metabolic engineering exploitation. Significant work has been carried out on this organism at the transcriptomic, and proteomic levels, with more recent studies also analysing metabolic pathways and products. In this study, we sought to analyse the transient response of *S.* sp. PCC6803 to alterations in light level. *S.* sp. PCC6803 was grown in a photobioreactor at 28°C and varying light cycles of sub 1 hour light:dark through to complete darkness, thus stimulating heterotropic metabolism. To assess the physiology of *S.* sp. PCC6803, mRNA and total protein were obtained at different stages in the growth cycle. Protein variations were analysed using 2-D electrophoresis, using broad range (pH 3–10) IEF strips. Over 200 spots were generated per phenotype, with differential changes assessed using PDQuest. Analysis of trypsin-generated peptide tags from differentially expressed proteins, and 50 common proteins was undertaken using an electrospray ionization quadrupole time of flight mass spectrometer (QStar XL, Applied Biosystems) equipped with capillary liquid chromatography (LC Packings). Peptide mass fingerprints from MS and MS-MS studies were analysed using Mascot software against the whole non-redundant protein database at NCBI. Furthermore, gels were also compared to Cyano2Dbase maintained at Kazusa. Many proteins were related to photosynthetic apparatus-like pigments, such as phycocyanin and allophycocanin, and proteins involved in the respiratory electron transport pathway. Gas and liquid chromatography, coupled to mass spectrometry, was used, where appropriate, to measure metabolic products. Particular attention was paid to products of central metabolism and those of secondary metabolism, such as H_2. Here, we analyse the response time scales for the oscillatory metabolic networks induced.

PBMG 11 A proteomic analysis of the alcohol metabolism of *Sulfolobus solfataricus*

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Sulfolobus solfataricus, a thermocidophilic sulphur metabolising crenarcheon, has been characterised with 14 putative alcohol dehydrogenases (ADHs). Primers for each ADH were designed and tested using RT-PCR with mRNA extracted from *Sulfolobus solfataricus* P2 grown at 80°C in a basal medium supplemented with glucose and 0.2–0.5% 2-propanol (iso-propanol), 1-propanol, ethanol, phenol and hexanol to identify the corresponding ADH genes involved. In parallel, a proteomic study of *S. solfataricus* grown in the presence of the aforementioned alcohols was carried out. Proteins were extracted from the cultures, and separated using 1D SDS-Page and large format 2-D electrophoresis with both narrow range and broad range IEF strips. Around 300 proteins were generated per 2-D gel. Comparisons were done between the differentially expressed proteins generated in cultures grown in presence and absence of various alcohols. The differences in the phenotypes were assessed using PDQuest software to determine presence vs absence and expression level. Differentially expressed spots were excised and digested with trysin. Subsequently, these peptide tags were identified using an electrospray ionisation quadrupole time of flight mass spectrometer (QStarXL, Applied Biosystems) fitted with a capillary liquid chromatograph (LC Packings). MS and MS-MS spectra were analysed using Mascot and the whole non-redundant protein database moderated at NCBI. Typically 23–75% protein coverage was obtained against predicted *S. solfataricus* proteins. These analyses allow for alcohol pathway reconstruction. With this information, future ADH metabolic engineering strategies can be realised to potentially solve industrial environmental issues, or for chiral alcohol synthesis.

PBMG 12 High-throughput prokaryotic and viral mapping and resequencing using custom high-density microarrays

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We have developed a strategy for high-throughput resequencing of prokaryotic organisms and viruses on high-density oligonucleotide microarrays. Using Maskless Array Synthesis (MAS) technology, we synthesize arrays with >380,000 different oligonucleotides, which are then hybridized with the entire genome of interest, and the sequence is read. Greater than 48,000 bases can be sequenced in a single array hybridization, and one technician can hybridize 40 arrays in a 24-hour period without automation, resulting in nearly 2 Mbases of sequence. Sequence call rates are typically >99%, with concordance of >99.99% with ABI capillary sequencing. This strategy has several advantages over traditional sequencing techniques. It eliminates the need to perform PCR on selected regions of the genome and the arduous task of sequence alignment, because the sequence is simply read directly from the arrays and concatenated. This strategy has been employed to resequence the entire 4.8 Mbase genome of a variant of *Salmonella enterica*, the genomes of over 14 clinical isolates of the SARS virus, as well as portions of the *E. coli* K12 and 0157:H7 genomes. Combining this resequencing strategy with an array-based whole genome mapping approach, it is possible to identify and sequence mutations at a rate of 20 Mbases per technician per day without automation. Leveraging this throughput with the flexibility of array design provided by MAS technology enables cost-effective, high-throughout mutation analysis and comparative genomics at the single nucleotide level in any prokaryotic organism or virus for which there exists a sequenced reference genome.

PBMG 13 Connections between the control of translation initiation and the mRNA decay process

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Global protein synthesis in yeast is rapidly regulated at the initiation step following a variety of nutritional and environmental stresses. Recently, we have found that mutants in the mRNA decapping process and the pathway of 60S ribosomal subunit biogenesis are resistant to many of these global translational controls. One of the regulated steps of translation initiation involves the formation of an eIF2 GTP Met-tRNA^i ternary complex. Curiously, the stress-dependent regulation of ternary complex levels is maintained in our resistant mutants. As all of these mutants are known to accumulate specific components of the protein synthetic machinery, we propose that this accumulation of translational apparatus may drive translation initiation by mass action, allowing regulation to be bypassed.

Recently several mRNA decay factors were shown to localise to specific cytoplasmic foci. Intriguingly, in preliminary work we have found that translation initiation factors such as eIF2 and eIF2B, which are involved in ternary complex formation, localise similarly to defined regions of the cytoplasm. It is possible that the location of these protein complexes may relate to the regulation of translation and also may be connected to the resistant phenotype observed in mRNA decay mutants.

Posters

Plenary

PS 01 Ticks and tick-borne micro-organisms in Slovakia

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The prevalence of ticks in different localities, infestation of ticks with *Rickettsia sp.* and *C. burnetii*, the mutual co-infection of these bacteria and with *A. phagocytophila* and *B. burgdorferi* s. l. and the preferences of bacteria to their vectors and hosts were evaluated in the current study.

A total of 7,527 adult ticks of four species: *I. ricinus, D. reticulatus, D. marginatus,* and *H. concinna* were collected by flagging the vegetation in 37 localities of Slovakia during the years 1999–2001.

The highest occurrence was achieved by *I. ricinus* in the lowland woodlands of eastern and western Slovakia.

By the haemocyte test (HT) the most infected were *D. marginatus* ticks, the frequency was 24.03%.

By PCR-RFLP *Rickettsia sp.* was confirmed in 41 *I. ricinus* and 15 *D. marginatus. C. burnetii* was found in one *I. ricinus* and one *H. concinna*. The presence of *A. phagocytophila* was found in 6 *I. ricinus* and one *H. concinna* ticks. *B. afzelii* was confirmed in 4 *I. ricinus* ticks, *B. garinii* in one *I. ricinus*. Co-infection of *Rickettsia sp.* with *A. phagocytophila* was recorded in one *I. ricinus* tick originated from Martinsky forest, western Slovakia.

The difference between the results of the HT and PCR was statistically significant.

A total of 45 spleens of live-trapped small mammals examined by Gimenez staining were negative to rickettsiae and coxiellae. By PCR *Rickettsia* sp. was found in one spleen of *A. flavicollis* and one spleen of *C. glareolus*; *C. burnetii* was identified in one spleen of *C. glareolus* only; *A. phagocytophila* in 2 spleens of *A. flavicollis* and 2 spleens of *C. glareolus.*

Based on the results we conclude that the localities under study are potential foci of tick-transmitted diseases. Ticks species such as *I. ricinus* are probably the main vectors for these micro-organisms and *A. flavicollis, C. glareolus* seem to be the hosts.

Supported by VEGA 2/1059 and NATO 15196/02B/TB. Dr Spitalska is currently a Royal Society NATO Postdoctoral Fellow.

PS 02 Molecular detection of a new tick-borne *Theileria* species in Red Hartebeest (*Alcelaphus buselaphus*) from Namibia

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Blood and tick samples from Red hartebeest antelopes were collected during the summer 2003 in Namibia. 22 out of the 23 blood samples were positively amplified by PCR based on the amplification of a 520bp fragment on the 18S rRNA gene. This PCR reaction is specific for the species belonging to the Piroplasmidae family (Theileria and Babesia species only). The size of the amplified fragment proved it was a Theileria species (as Babesia species show only a 450bp fragment). Sequencing was performed on four isolates and a similar sequence was observed for each isolate. A GenBank comparison showed no matching (at least 90bps are different between our isolate and the Genbank sequences) with the usual African Theileria species such as *Theileria parva, T. mutans, T. taurotragi* or *T. velifera*. However our DNA sequences matched a *Theileria sp.* sequence published in 1994 by Allsopp *et al.*, considering the high prevalence (96%) and the lack of clinical signs on these animals it seems we are dealing here with a new mild species of Theileria. Future work will try to identify the tick vector responsible for the transmission to these antelopes and if other ruminants are infected with this new species.

Acknowledgements: Dr Eva Spitalska is financially supported by a Royal Society Nato Postdoctoral Fellowship and is a visiting scientist from the Slovak Academy of Sciences from Bratislava, Slovakia.

Reference: Allsopp *et al.* (1994). *Parasitology* **108**, 147–152.

PS 03 Prevalence of *Borrelia burgdorferi sensu lato* infection and intensity of infection in female taiga ticks, *Ixodes persulcatus* Schulze (Acari: Ixodidae), of different physiological age

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It has been accepted that the relationships between arthropod vectors and the microbial pathogens they transmit are of a parasitic nature. In some studies the prevalence of vector infection and the intensity of infection are negatively correlated with vector age (in the broad sense of this term). However, no special research has been carried out to consider the phenomenon thoroughly. The infection indices of the female taiga ticks with *Borrelia burgdorferi* s.l. were compared with tick physiological age, an index that more precisely reflects tick physiological state than the time of tick collection or the duration of tick life under laboratory conditions. The physiological age was determined by a novel quantitative technique. In total, 131 female ticks were examined for their infection and physiological age, 45 of which were infected with *B. burgdorferi* s.l. (infection rate 34.4%). The minimal intensity of infection was 0.4 bacterial cells per 100 fields of view whereas the maximal infection was 172 cells. A statistically significant difference between both the prevalence of infection and intensity of infection in ticks of different physiological age was not found. However, the number of physiologically young as well as very old ticks was not so large as to consider the data final. On the other hand, the specificity of *Borrelia* behaviour in the tick midgut makes it possible to accept the data obtained and to create a model of the relationship between ticks and pathogens during tick ageing.

PS 04 Characterisation of a novel protein with possible function in *Yersinia pestis* colonisation of the flea midgut

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Enhancins are a class of metalloproteases found in some baculoviruses that enhance viral infection by degrading the

peritrophic membrane (PM) of the insect midgut, exposing the underlying epithelial cells to infection. The only known substrate for viral Enhancin is insect intestinal mucin (IIM), a 400 kDa glycoprotein within the PM. A gene with 24% homology to viral *enhancin* was found in the recently published genome sequence of *Yersinia pestis* and the bacterium may have acquired the gene from an insect virus by horizontal transfer. The bacterial Enhancin (YpE) may help *Y. pestis* colonise the midgut of the flea in a similar manner to baculovirus Enhancin. The *enhancin* gene (Ypo0339) was PCR amplified from *Yersinia pseudotuberculosis*, (closely related to *Y. pestis*) and cloned into the expression vector pTriEx for use in the baculovirus expression system. A recombinant *Autographa californica* multiple nucleopolyhedrosis virus (*Ac*MNPV), which does not encode an *enhancin* gene, was generated to express YpE. Nucleotide sequence alignments showed it to be identical to the *Y. pestis* sequence. Amino acid alignments of viral and bacterial Enhancins showed a conserved zinc binding motif (HEXXH) with highest homology in the N-terminal region. Western blots show YpE to be a 95–100 kDa protein. Soluble protein was purified from insect cells and assayed with IIM, no breakdown was observed. Recombinant virus expressing YpE exhibited higher cytotoxicity in tissue culture and killed infected larvae faster. These data suggest that YpE is active but may have lost IIM specificity since divergence from the viral homologue.

PS 05 The epidemiology of genetic exchange in *Trypanosoma cruzi*: triatomine vectors verse vertebrate hosts

Michael W. Gaunt, Matthew Yeo & Michael A. Miles

University College London

Trpanosoma cruzi is responsible for an estimated 11–13 million human infections in Latin America. Triatomine bugs are the sole vectors of *T. cruzi*, whilst a range of sylvatic mammals act as reservoir hosts. All infections persist for the lifespan of the host.

We have recently demonstrated the mechanism of genetic exchange in *T. cruzi* by observing strong genetic parallels between the experimental production of hybrid clones and the phylogenetic analysis of natural isolates. The mechanism is distinct from that proposed for the African trypanosome, *T. brucei* and instead involves aneuploidy through nuclear hybridization. We present the evidence that unlike *T. brucei*, the mammal and not the vector is the site of recombination in *T. cruzi*. Our evidence generally infers the vector is not the principle epidemiological source of biodiversity in *T. cruzi* and we discus the co-evolutionary relevance of our molecular clock studies on dating the origin of triatomine bugs (insect molecular clock) and previous work on dating the origin of the parasite.

PS 06 Pathogens in a field vole – multiple-tick system

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Woodland rodents have been shown to be important reservoirs of tick-borne infections throughout the world. However, in the UK, as woodland rodents carry only very few infectious life stages of *Ixodes ricinus*, the principal vector of tick-borne diseases in Europe, their role in the epidemiology of tick-borne infections has been deemed relatively unimportant. We report data from a field study in Kielder Forest in Northumberland where *I. ricinus* and a second tick species, *Ixodes trianguliceps*, are present. Although *I. trianguliceps* is implicated in the natural cycles of the human pathogens *Anaplasma phagocytophilum* and *Babesia microti*, all life stages of the tick feed solely on small rodents thus pose little threat as transmitters of disease to humans. Data from this ongoing study show that individual field voles are hosts to both tick species concurrently, and are infected with both *A. phagocytophilum* and *B. microti*. We hypothesise that *I. ricinus* may act as a bridge vector, enabling infections maintained in an enzootic vole-*I. trianguliceps* cycle to 'escape' and pose a risk to humans and domesticated animals. As such, the role of rodents in the epidemiology of tick-borne infections in the UK may need to be re-assessed.

Posters

Systematics & Evolution Group

SE 01 Characterisation of novel *Nonomuraea* strain from Canary Basin sediment

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The genus *Nonomuraea* was proposed by Zhang *et al.* (1998) to accommodate actinomycetes which form an extensively branched substrate and aerial mycelium. The taxon currently encompasses eighteen validly described species which form a distinct phyletic line within the evolutionary radiation occupied by members of the family *Streptosporangiaceae*. In the search for rare and novel actinomycetes, which may prove to be the source of new and effective therapeutic natural products, including antibiotics, an actinomycete designated as CB17-055, was isolated from sediment collected from the Canary Basin. Phylogenetic analysis based on a 16S rRNA gene sequence showed that this isolate belongs to genus *Nonomuraea*, forming a subclade with *Nonomuraea roseoviolacea*. Strain CB17-055 can be distinguished from the type strains of all the validly described species of *Nonomuraea* using a combination of phenotypic properties. The genotypic and phenotypic data indicate that the isolate should be recognised as a new species of *Nonomuraea*. This is the first report of the isolation of a *Nonomuraea* strain from marine environment.

SE 02 Abstract withdrawn

SE 03 Development of an insect model of infection by the important human pathogen *Staphylococcus aureus*

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Staphylococcus aureus represents a major cause of community-acquired and nosocomial sepsis. Recent evidence suggests bacterial virulence is determined by the total number of virulence genes present in a given strain. In order to examine natural variation in virulence potential, we have developed an *in vivo* model based on the insect larvae *Manduca sexta*. Preliminary research suggests an LD_{50} of approximately 10^4 cells of *S. aureus* at 37°C, with viable *S. aureus* cells readily recovered from the cadaver, suggesting that *in vivo* colonisation has occurred. Through the use of a well-characterised bacterial strain collection, we have demonstrated consistent differences in virulence (as measured by weight change in the larvae) between bacterial strains, and noted correlations with the strain-specific virulence gene-profile. Those strains which have most commonly been recovered from cases of serious disease in humans tend to show the greatest virulence in *M. sexta*, which indicates great promise for insect models of virulence for human pathogens.

SE 04 Recombination and sequence evolution within the natural population of *Staphylococcus aureus*

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Staphylococcus aureus is an important opportunistic human pathogen and represents an increasing public-health burden owing to the spread of drug-resistant clones within the hospital environment. Multi-locus sequence typing (MLST) is a powerful typing scheme which can be used for local and global epidemiological surveillance, and these data also provide evidence that recombination is relatively rare in *S. aureus*. We have used existing MLST data as a framework to sample 26 diverse staphylococcal genotypes, recovered both from invasive disease and asymptomatic carriage, and have extended the MLST data to include approximately 37 further loci. These loci have been chosen to represent both diverse functional classes and physical location on the chromosome, and these data provide evidence regarding the phylogenetic consistency, and relative rates of recombination, around the genome. Since recombination is relatively rare in *S. aureus*, the reconstruction of a consensus intra-specific phylogenetic tree is feasible.

SE 05 Sequence diversity within SdrE: a staphylococcal surface adhesin

J.E. Cooper, N.P.J. Day, S.H. Peacock, H. Grundman & E.J. Feil

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The completion of seven *Staphylococcus aureus* genomes has revealed the presence of a large number of genes encoding adhesions and toxins which potentially play a role in the virulence of this bacterium. One of these, *sdr*E, forms part of a family of three related adhesin genes, *sdr*C, *sdr*D and *sdr*E, found tightly linked within a ~12-Kb region of the genome. Two quite distinct allelic variants are found within natural populations at this locus. One of these variants corresponds to the *bbp* gene previously assumed to be a different gene). Furthermore, a PCR assay of a large strain collection (n=452) revealed that the presence of the *sdr*E-like variant, rather than the *bbp*-like variant, at this locus was significantly more frequently observed in isolates recovered from invasive disease than in isolates recovered from asymptomatic carriage ($p< 0.001$). This raises the possibility that the presence of one or other of these different variants influences the probability that colonization by *S. aureus* will subsequently cause invasive disease. To investigate this further we have sequenced the entire *sdr*E/*bbp* gene (~3-Kb) in 16 *S. aureus* isolates representing both disease and asymptomatic carriage to characterize the differences between and within these variants at the molecular level.

SE 06 Analysis of the mutational profile of *S. aureus* genomes

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Seven complete genome sequences are available for *S. aureus*. By placing the sequenced strains into a population framework provided by multilocus sequence data, the seven sequences are divided into three pairs (in which both isolates belong to the same clone or clonal complex) and one sequence from a distinct clonal complex. Sequences from the same clonal complex have diverged very recently and the core genomes differ by 200–300 SNPs in each case. An excess of non-synonymous substitutions is noted in closely related genomes, which probably reflects insufficient time for purifying selection to remove slightly deleterious mutations from the population. These SNPs therefore approach the mutational profile of the genome, and by using the single diverse sequence as an outgroup

it is possible to assign directionality to each point mutation. The resulting data suggest that at mutational equilibrium the GC content of this species would be approximately 22%, much lower than the 33% observed. Either the GC content of this species is continuing to fall, or selection is maintaining it at is present level.

SE 07 Evolution of the accessory gene regulator (*agr*) in natural populations of *Staphylococcus aureus*

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The virulon of *Staphylococcus aureus* is regulated in part by the accessory gene regulator (*agr*), a two-component signal transduction system. Four alleles of the autoinducing peptide pheromone, *agrD*, have been characterized in *S. aureus*. The cross-inhibitory effects of the *agrD* alleles have been offered as a biological basis for subdividing the species. To test whether the biological subdivisions of *agr* reflect the phylogenetic subdivisions within the species, we determined the *agrD* allele by PCR for a set of 220 diverse isolates for which portions of 15 genes had been previously sequenced. By mapping the *agrD* alleles onto a total-evidence phylogenetic tree, we find that most isolates of the same clonal complex have the same *agrD* allele. The hypothesis of occasional recombination events at the *agr* locus was tested by sequencing in and around the *agr* locus. Non-parametric bootstrapping of a total-evidence phylogenetic tree suggested that broader groups of clonal complexes can have different *agrD* alleles. This phylogenetic hypothesis was tested with parametric bootstrap analyses. We conclude that recombination events at the *agr* locus have occurred and, therefore, the biological subdivision of the species on the basis of *agr* does not necessarily reflect a natural phylogenetic subdivision.

SE 08 Rapid detection of *Escherichia coli* using screen-printed carbon electrodes

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Hydrogen peroxide (H_2O_2) can be detected electrochemically, therefore catalase activity of aerobes and facultative anaerobes may be monitored via the enzymatic degradation of a fixed concentration of H_2O_2, and related to bacterial cell numbers. Sippy *et al.* (2003) previously demonstrated the detection of *E. coli* in the range $2x10^1$–$2x10^7$ cfu/ml, using capture by lateral flow immunoassay (LFI) and detection at a platinum electrode. Modification of this method to use inexpensive, disposable screen-printed carbon electrodes (SPCE) instead of the platinum electrode could enable production of a hand-held device that could be used in a number of fields (e.g. the food industry, environmental monitoring, biomedicine).

SPCE consist of a carbon ink working electrode and a silver/silver chloride reference electrode printed on to a poly vinyl chloride (PVC) strip. Addition of 5% cobalt phthalocyanine (CoPC) electrocatalyst to the carbon ink lowers the potential needed for the oxidation of H_2O_2 from +0.7V to +0.42V (vs. Ag/AgCl), increasing selectivity. Bacterial cell suspensions and H_2O_2 were applied directly to the SPCE surface and *E. coli* was detectable at $3x10^5$ cfu/ml, after an incubation time of only 3 minutes. Further improvement of the sensitivity will be possible by modifying the electrochemical technique.

Authors

Plenary session

Microbe–vector interactions in vector-borne diseases

Reducing the prevalence of *Borrelia* in ticks

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The risk for humans of Lyme borreliosis (LB) is a function of the prevalence of *Ixodes* species nymphal ticks infected with the disease agent. To lower the incidence of LB, individuals in endemic areas could be immunized against *Borrelia burgdorferi* (Bb), but the risk would remain the same. Among strategies to reduce the density of infected ticks are those that would reduce the overall number of ticks; these include pesticides and removal of certain critical host mammals. Another approach is to target a transmission-blocking vaccine to those vertebrates that are both competent reservoirs of Bb and the hosts for larvae of *Ixodes* sp. The end-point for a field vaccination trial is a comparison of vaccine and control grids for the prevalences of Bb infection among tick nymphs the following year. For this aim, we carried out a controlled trial in Connecticut, USA, and used recombinant OspA lipoprotein as the vaccine, *Peromyscus leucopus* mice as the targeted reservoir, and quantitation of Bb infection in *I. scapularis* nymphs. In addition, we determined the population structure of Bb in both ticks and reservoirs at the field site and investigated the dynamics of infection and immune response among the mice.

Posters

Physiology, Biochemistry & Molecular Genetics Group

PBMG 14 Key structural elements of VA RNA_I

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VA RNA_I, produced by all adenoviruses, is ~160 nucleotides in length and has a secondary structure predicted by mutational and probing analyses that consists of two duplexed stems (apical and terminal) joined in the centre by a complex stem loop structure. VA RNA_I blocks the action of PKR, preventing the binding of activating dsRNA molecules. This inhibition prevents the block on translation, created through the phosphorylation of eIF2α by PKR, thereby allowing the completion of the viral growth cycle.

Here we are concentrating on the key structural elements of VA RNA_I that confer the ability to bind and inhibit PKR. Previous mutational research has mainly focused on the central domain and apical stem, in which generally large deletions or linker scanning mutations have been made. The mutational approach undertaken here is to create small deletion mutations (~5 bp) to try and identify the key functional areas of the RNA while keeping disruption the secondary structure to a minimum. The structure and function of these mutants will be assessed using UV melting analysis in combination with PKR inhibition assays.

VA RNA_I unfolds in two apparent transitions with the terminal stem and central domain unfolding first (T_m 62°C), and the apical stem unfolding at a slightly higher temperature (T_m 86°C). The initial deletion mutations were made in the terminal stem of the RNA, creating a gradual shortening of the stem. From the UV melting analysis of these mutants the first peak of the profile is affected, confirming that the terminal stem unfolds first. Further mutations were then made at the top of the apical stem. These mutants shifted the second peak to a higher melting temperature, suggesting a stabilising of the RNA. From the inhibition assays with PKR the apical stem mutants appear to have little effect on the RNAs functional ability, whereas the larger terminal stem mutations seem to show a decrease in inhibition.

PBMG 15 Assembly of MFC and 40S/eIF complexes *in vitro* for investigation by Cryo-EM

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In order to elucidate the mechanisms underlying translation initiation event in eukaryotes we have set out to obtain structural information about ribosomal-protein complexes involved in different steps of this process. The 40S ribosomal subunit is prepared for the recruitment step by interaction with a group of eukaryotic initiation factors (eIFs) that, at least in budding yeast, forms a multifactor complex (MFC). A number of translation initiation complexes assembled from individual translational components were prepared for analysis by Cryo-EM and other biophysical methods.

40S ribosomal subunits were prepared from *Saccharomyces cerevisiae*. The MFC and 40S/eIF complexes were formed by mixing equimolar amounts of the eIFs in 3-fold excess of 40S subunits. 43S complexes, comprising eIF1, eIF-1A, Met-tRNAiMet.eIF2.GTP, eIF3 and eIF5, were formed. All the complexes were separated by sucrose gradient centrifugation and the fractions in the sucrose gradient peak were analysed by means of Western blotting.

Cryo-EM reconstructions reveal the positioning of the MFC factors on the 40S subunit and the occurrence of major conformational changes in the 40S subunit, including large rotational movements of the head and platform, upon MFC binding. These and other data will be presented in the poster.

PBMG 16 Force-distance curves for RNA unwinding and the influence of helicases

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RNA helicases are essential in a wide range of biological processes. One example of a DEAD box family RNA helicase is eIF4A, which is a eukaryotic translation initiation factor that plays a vital role in removing secondary structure from the 5′ UTR of mRNAs, hence aiding the binding and subsequent scanning of the 40S ribosomal subunit. Another example is the Hepatitus C Virus (HCV) Non-structural protein-3 (HCV-NS3) helicase domain that is thought to act processively in separating the double stranded HCV RNA genome intermediate during viral replication and so is an important anti-viral drug target. Despite extensive biochemical studies, little is still known about the details of RNA helicase dynamics.

The activity and mechanism of action of DEAD box helicases can be investigated directly by means of a novel force spectroscopy assay using the Atomic Force Microscope (AFM). Here, an RNA molecule containing secondary structure is bound to a gold-coated slide via a 5′ terminal thiol modification. The 3′ end of the molecule has a biotinylated poly(A) tail that can be picked up by a streptavidin-coated AFM tip. Stretching this construct generates force-extension curves that describe the force required to remove secondary structure from the RNA. Addition of a helicase to the system should partially

unwind the dsRNA region and reduce the force required to stretch out the RNA. This poster describes how the strategy outlined here is realised in practice and presents force-distance curves for some of the experiments that have been performed.

This work is supported by the BBSRC.

PBMG 17 eIF4E2 is a novel type of stress response protein

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In contrast to *Sacchoromyces cerevisiae*, *Schizosaccharomyces pombe* has two eIF4E genes. The presence of more than one eIF4E-type gene in genome is typical of higher organisms including man (1).

Cap-binding proteins of the eIF4E family are generally involved in mediating ribosome recruitment to capped mRNA via an interaction with the initiation factor eIF4G. However, *Schizosaccharomyces pombe* (2) has two eIF4E isoforms, one of which (eIF4E2, encoded by *tif452*) has a relatively low affinity for eIF4G. We show that *tif452* is required for specific stress responses. An *S. pombe tif452*Δ mutant manifests slow growth under conditions of nutrient, temperature and salt stress. In response to salt stress, the cellular level of eIF4E2 increases while the amount of intact eIF4G decreases, leaving eIF4E2 as the predominant eIF4E isoform in a cell deficient in eIF4G. The presence of eIF4E2 modifies the competence of *S.pombe* ribosomes to translate mRNAs with structured leaders *in vivo*.

The *tif452* promoter has putative stress-response (T-rich) motifs while eIF4E2 seems to be a new type of stress-response factor (3).

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