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Full chapters of the following presentations will be published in a Symposium Volume “*Fighting infection in the 21st Century*” published for the Society General for Microbiology and the Society for Applied Microbiology by Blackwells.

MONDAY 10 APRIL 2000

- 0945 The global threat of emerging infectious diseases**
B.W.J. MAHY (CDC, Atlanta, USA)
- 1115 Can molecular techniques be used in the prevention of contamination of processed food by pathogens?**
T.J. HUMPHREY (PHLS, Exeter)
- 1200 Is global clean water attainable?**
J. BARTRAM (WHO, Geneva, Switzerland)
- 1345 Vaccine development: past, present and future**
C. HENDRIKSEN (Rijksinstituut voor volksgezondheid en milieu, Bilthoven, The Netherlands)
- 1430 Live attenuated vectors: have they delivered?**
M.M. LEVINE (University of Maryland, USA)
- 1545 New malaria vaccines: the DNA–MVA prime–boost strategy**
A.V.S. HILL (University of Oxford)

TUESDAY 11 APRIL 2000

- 0900 Prospects for a new and a rediscovered form of therapy: probiotics and phage**
S. BÈNGMARK (Ideon Research Centre, Lund, Sweden)
- 0945 Vaccine production in plants**
G.C. WHITELAM (University of Leicester)
- 1115 Immunotherapy of sepsis – is there a future?**
E.T. RIETSCHÉL (Research Center Borstel, Germany)
- 1200 Bioterrorism**
P. TAYLOR (CBD Porton Down)
- 1345 Characterization of bacterial isolates with molecular techniques: multi locus sequence typing**
M.C.J. MAIDEN (University of Oxford)
- 1430 The world-wide epidemic of antibiotic resistant bacteria: do antibiotics have a future?**
A. TOMASZ (Rockefeller University, USA)
- 1545 New strategies for identifying and developing novel vaccines: genome-based discoveries**
L. LISSOLO (Pasteur Aventis, Marcy l’Etoile, France)
- 1630 Are molecular methods the optimum route to antimicrobial drugs?**
F.C. ODDS (University of Aberdeen, UK)

THURSDAY 13 APRIL 2000

0900 Regulation by proteolysis in prokaryotes

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Construction of a finely tuned and rapidly responsive regulatory network or proper timing of a developmental pathway frequently requires the control of degradation as well as synthesis of key components. In prokaryotes, much of the critical proteolysis is dependent on a handful of energy-dependent cytoplasmic proteases. Proteolytic sites are sequestered in a chamber not directly accessible to folded proteins; appropriate substrates are first recognized by ATPase domains and then unfolded and translocated into the proteolytic chamber where proteolysis is rapid and processive. In cases where proteolysis of a given substrate is itself regulated under different environmental conditions, additional factors may provide the regulation mechanism. Best understood of these systems is the degradation of the *E. coli* stationary sigma factor, RpoS. Degradation is carried out by the ClpXP protease, but only when an additional protein, RssB (SprE) is present. RssB, although not itself subject to degradation, is necessary for ClpX, the ATPase component of this protease to recognize and bind RpoS. The activity of RssB is modulated by the reversible phosphorylation of the amino-terminal domain, which has the characteristics of a response regulator.

0935 The pathway of substrate movement through the proteasome

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The proteasome is formed by the association of a core particle (CP) and a regulatory particle (RP). The RP is composed of the 8-subunit lid and base assemblies. The proteolytic active sites of the proteasome are contained within the luminal space of the CP, suggesting that substrates must be unfolded before being translocated through a channel into the CP lumen. Proteasomes exhibited an ATP-dependent chaperone-like activity, which was mapped to the base. Moreover, the base can bind denatured proteins in a ubiquitin-independent fashion. We thus propose that substrates are tethered to the proteasome via recognition of their ubiquitin chains, followed by an interaction between the base and the target protein, which is coupled to unfolding and translocation of the target protein into the CP.

The crystal structure of the free CP shows no evidence of a substrate channel. Free CP has little activity against peptides, but is stimulated in the presence of RP. Deletion of the N-terminus of the alpha3 subunit resulted in constitutive derepression of the free CP for peptide hydrolysis. The crystal structure of the CP from the alpha3 deletant revealed that the mutation resulted in the formation of an axial channel into the CP, thus allowing rapid entry of peptides into the lumen. In summary, 1) there is a channel in the CP, allowing for

hydrolysis by the CP is limited by substrate entry, 4) classical "activation" of the CP reflects channel opening, 5) the channel is opened when the base contacts the CP.

The chaperone site and substrate channel presumably represent the final two stations for substrates as they migrate into the lumen of the CP for hydrolysis.

1010 Functions and mechanisms of ubiquitin-dependent protein degradation

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Proteins modified by multiubiquitin chains are the preferred substrates of the proteasome. Ubiquitination involves a ubiquitin-activating enzyme E1, a ubiquitin-conjugating enzyme E2, and often a substrate-specific ubiquitin-protein ligase E3. Recently, we identified several new factors involved in ubiquitin/proteasome-dependent proteolysis. One of these factors, which we termed E4, binds to the ubiquitin moieties of preformed conjugates and catalyzes ubiquitin chain assembly in conjunction with E1, E2, and E3. E4 interacts with CDC48, a putative chaperone of the AAA type ATPase family. We identified several additional interacting proteins of CDC48 by various screens. One of these proteins appears to be a conserved co-factor of CDC48 and mediates proteasome-dependent functions. The specific functions of the new proteolysis factors will be discussed.

1115 Cellular proteases involved in virus maturation

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The surface glycoproteins of many enveloped viruses undergo posttranslational cleavage by host proteases at arginine containing cleavage sites. These glycoproteins are often fusion proteins, and cleavage has been shown to be necessary for fusion activity. Proteolytic activation, which depends on the structure of the cleavage site and the availability of an appropriate protease, is a determinant for spread of infection and pathogenicity. Important cleavage enzymes are furin and other members of the proprotein convertase family that recognize multibasic cleavage sites. These proteases are TGN residents and cleave the nascent viral glycoprotein during exocytotic transport. Other cleavage enzymes are secreted, such as factor X, tryptase Clara, plasmin, and bacterial proteases. They cleave at monobasic cleavage sites. Cleavage may also occur after virus entry in endocytotic vesicles. Recent studies on Ebola virus have shown that a secreted viral glycoprotein is also cleaved by furin and that the ectodomain of the envelope glycoprotein of this virus can be removed, presumably by a secretase.

1150 Secreted aspartyl proteinases as virulence determinants in *Candida albicans*

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The medically important fungal pathogen *Candida albicans* often causes superficial infections of mucosa and skin or even life-threatening systemic infections in immunocompromised patients. One of the possible virulence attributes is

All SAPs are translated into prepropeptides with at least one Lys-Arg or Lys-Lys processing site for Kex2-like regulatory proteinases. The mature proteinases contain conserved aspartate residues at the active sites and conserved cysteine residues.

At the transcriptional level, members of the *SAP* gene family are differentially regulated both *in vitro* and *in vivo*. Distinct *SAP* genes are expressed during mucosal and disseminated infections and were shown to be of different importance according to the type of infection.

For example, in a model of oral candidiasis using reconstituted human epithelia we observed a temporal progression of *SAP* expression in the order *SAP1* and *SAP3* > *SAP6* > *SAP2* and *SAP8* which correlated with increasing tissue damage. Similar expression patterns of *SAP* genes were also detected by RT-PCR in samples from patients suffering from oral candidiasis. To determine whether Sap activity was responsible for the observed tissue damage, specific *SAP* gene deletion mutants were constructed and evaluated in the oral candidiasis model. A comparison of these single, double and triple mutants with the corresponding wild type *C. albicans* strain showed that *SAP1-3* are clearly involved in tissue damage during experimental oral candidiasis. In contrast, deletion of *SAP4-6* did not attenuate the histological lesions. Interestingly, disruption of *SAP1* and *3* caused up-regulation of *SAP8* in the model, suggesting that *C. albicans* may compensate for the deletion of certain *SAP* genes by up-regulation of alternative *SAP* genes.

1400 Regulated proteolysis of the RNA polymerase subunit RpoS, a master regulator of the general stress response in *E. coli*

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In *E. coli*, the promoter-recognizing sigma subunits of RNA polymerase, that are involved in acute stress responses, are controlled by proteolysis. A prime example is RpoS (sigma-S), which is the master regulator of the general stress response. Various stress conditions result in strong induction of RpoS by inhibiting proteolysis of RpoS, which is a highly unstable protein in non-stressed cells. RpoS proteolysis requires the "turnover element" within RpoS, ClpXP protease, and RssB, a two-component-type response regulator. In-vitro experiments with purified components demonstrated that RssB serves as a direct RpoS recognition factor whose affinity for RpoS is modulated by phosphorylation of its N-terminal receiver domain. The turnover element within RpoS represents the binding site for RssB. Its location in the middle of RpoS is highly unusual for a proteolysis recognition site. Moreover, the RssB binding site also overlaps with region 2.4/2.5, which is crucial for promoter recognition by RpoS (with K173 being an essential amino acid for both functions). RssB probably transfers RpoS to ClpXP protease, but is not co-degraded, suggesting a catalytic role for RssB in the initiation of RpoS proteolysis. Consistent with this, the cellular level of RssB is more than 20fold lower than that of RpoS. Stresses that affect RpoS proteolysis do not alter RssB levels, but rather may control its activity, probably by dephosphorylation. Under conditions of RssB overproduction and low ClpXP activity, RssB can also inhibit RpoS activity, suggesting that from an evolutionary perspective, RssB may have been a former anti-sigma factor, that was recruited by the proteolytic machinery to serve as a substrate recognition factor.

1435 ER quality control: selective protein export to the cytosol

The folding of secretory proteins in the endoplasmic reticulum (ER) is monitored by a complex and as yet poorly defined quality control mechanism that involves ER-resident chaperones. Proteins which fail quality control are not allowed to enter ER-to-Golgi transport vesicles, but rather are retained in the ER, and many are subsequently exported across the ER membrane back to the cytosol where they are degraded by proteasomes. Retrograde transport across the ER membrane is mediated by a channel containing Sec61p, the core-component of the channel responsible for secretory protein import into the ER. Other components of the export channel are unknown so far, but several additional ER transmembrane proteins required for ER degradation have been identified in genetic screens. Export may be driven by a Brownian ratchet mechanism similar to that employed for posttranslational protein import into the ER, with cytosolic chaperones or ubiquitination constituting the ratchet. The mechanisms of recognition of misfolded proteins in the ER lumen and of channel opening for export in the absence of a signal sequence remain to be elucidated.

1540 Role of cleaved signal peptides as biological messengers

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Export signal sequences target newly synthesized secretory and membrane proteins to the endoplasmic reticulum of eucaryotic cells and the plasma membrane of bacteria. All signal sequences contain a hydrophobic core region, but, despite this, they show great variation in both overall length and amino acid sequence. Recently, it has become clear that this variation allows signal sequences to specify different modes of targeting and membrane insertion and even perform functions after being cleaved from the parent protein. It becomes more and more evident that signal sequences are not simply greasy peptides but sophisticated, multipurpose peptides containing a wealth of functional information.

Signal sequences of some proteins are exceptionally long. For some it has been shown that fragments generated by the cleavage of signal peptidase and signal peptide peptidase are released into the cytoplasm. For signal sequence fragments of preprolactin and HIV-1 gp160 envelope protein we have identified Calmodulin as the cytoplasmic target. Other signal sequence fragments have been shown by others to function in antigen presentation by MHC class I molecules. We will discuss the various roles signal sequences can have in targeting, membrane insertion and as peptides after they have been cleaved.

1615 Activation of a developmental sigma factor in *Bacillus subtilis* requires autoproteolysis of the SpoIVB serine peptidase

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SpoIVB is essential for intercompartmental signalling in the σ^K -checkpoint of *Bacillus subtilis*. SpoIVB is synthesised in the spore chamber and is the signal which activates proteolytic processing of pro- σ^K to its mature and active form σ^K .

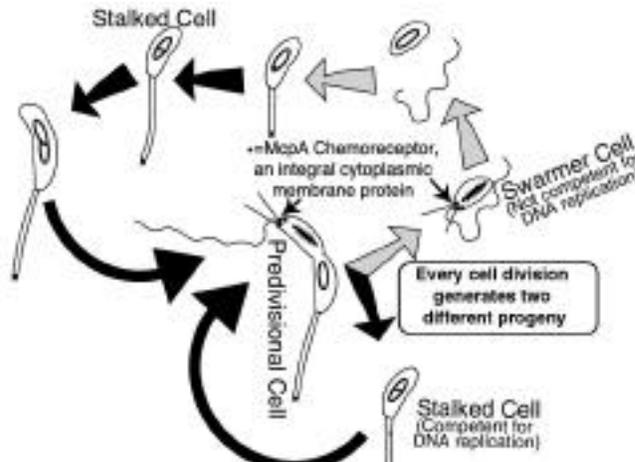
We show here that SpoIVB is a serine peptidase of the chymotrypsin SA clan. Expression of SpoIVB in *Escherichia coli* has shown that SpoIVB is able to cleave itself into at least three discrete cleavage products beginning at the N-terminus, and *in vitro* studies have shown cleavage to be *in trans*. Autoproteolysis of SpoIVB is essential for initiating the two developmental functions of this protein, signalling of pro- σ^K processing and a yet, uncharacterised, second function. In *Bacillus subtilis* SpoIVB is synthesised as a zymogen and is subject to two levels of proteolysis. Firstly, rapid and sequential autoproteolysis beginning at the N-terminus

switching off the active SpoIVB intermediates and suggests a similarity to other proteolytic cascades such as that found in blood coagulation.

FRIDAY 14 APRIL 2000

0900 Role of proteolysis in the cell-cycle regulation and asymmetric targeting of the chemotaxis machinery
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***Caulobacter crescentus* cell cycle**

The *Caulobacter crescentus* cytoplasmic membrane protein McpA is a member of a well-studied family of prokaryotic chemotaxis receptors. The McpA chemoreceptor is only synthesised in early predivisive cells where it is targeted to the cell pole opposite the stalk. Thus, once predivisive cells divide the chemoreceptors end up in the motile swarmer cell and not the sessile stalked cell. Although McpA is not synthesised in swarmer cells, it is stable until the cells start to differentiate into stalked cells when it is degraded. McpA is unstable in stalked cells. The amino acid sequence required for McpA degradation is located twelve residues from the carboxyl terminus, and thus unlike many ClpX dependent proteolysed substrates, McpA does not require the degradation signal to be located at the extreme C-terminus. The localization of McpA is not dependent on proteolysis as demonstrated by the polar localization of non-degraded derivatives of McpA. However, proteolysis is required for the generation of asymmetry of the chemoreceptor because non-degraded derivatives of McpA end up in both swarmer and stalked cells after cell division. Thus, proteolysis plays an important role in the spatial and temporal localization of the McpA chemoreceptor during the cell cycle in *C. crescentus*.

0935 Protection of cell cycle regulators from proteolysis

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DNA-integrity checkpoint pathways respond to changes in the DNA structure, detecting perturbations and transducing signals to the cell cycle machinery. In *S. pombe*, loss of any one of six "checkpoint Rad" proteins results in loss of all DNA-integrity checkpoints, including the G2-M and intra-S phase checkpoints after DNA damage and the S-M checkpoint during hydroxyurea treatment. Downstream of the checkpoint

localisation and the stability of the regulators of Cdc2 kinase in order to prevent entry into mitosis. During HU arrest, activation of the Cds1 results in Mik1 being stabilised. During the unperturbed cell cycle, Mik1 is stable during S phase, but degraded in G2. Surprisingly, this profile is not dependent on checkpoint Rad proteins or Cds1. However, maintaining Mik1 stability in HU arrested cells is dependent on Rad3 and Cds1. We propose that, upon checkpoint activation, Cds1 activity maintains an S phase status by negatively regulating the protein degradation machinery.

1010 The role of proteolysis in the *Caulobacter* life cycle

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In *Caulobacter crescentus* development is tightly coupled to the cell cycle. An asymmetric cell division gives rise to a motile but replication inert swarmer cell (SW) and a sessile, replication competent stalked cell (ST). While the ST cell immediately reinitiates a new round of replication, cell growth, and cell division, the SW cell performs chemotaxis for a defined period before it differentiates into a ST cell and starts a replicative cycle. To assess the significance of controlled protein synthesis and degradation for cell cycle progression we have established a comprehensive catalog of proteins that are subject to timed synthesis and degradation during the *C. crescentus* cell cycle. 15% of the proteins are differentially expressed during the cell cycle and about 5% are rapidly degraded. Surprisingly, more than half of the proteins, which are unstable are also expressed in a cell cycle-dependent manner. This implies that protein degradation plays a crucial role in bacterial cell cycle control. To understand temporal and spatial control of protein degradation during the *Caulobacter* cell cycle we study the role of the Clp protease in cell cycle progression and cell differentiation. Clp is a multi-component protease with a central ClpP peptidase chamber associated with either one of two interchangeable regulatory ATPases, ClpA or ClpX. We have found that in *Caulobacter* the Clp protease is involved in both cell cycle control and development and that the cell has recruited ClpX for cell cycle progression and ClpA for cell differentiation.

1115 Programmed proteolysis of cyclins during meiosis and mitosis

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Proteolysis of mitotic cyclins requires multiubiquitinylation catalysed by the E3 ubiquitin ligase known as the Anaphase Promoting Factor or Cyclosome (APC/C). The APC/C is activated by phosphorylation of its constituent subunits and by accessory factors of the fizzy/Cdc20 family, and is under the control of the spindle assembly checkpoint pathway. We want to understand how it is that cyclin A is degraded before cyclin B, and have investigated the proteolysis of cyclin A in human cells and frog egg extracts. We find that cyclin A proteolysis is not regulated by the normal checkpoint mechanism; its degradation immediately follows activation of the APC/C in prometaphase. Thus, cyclin A is unstable in CSF-arrested frog egg extracts, in nocodazole-treated cells, and in cells over-expressing Mad2. By contrast, cyclin A is stable in cells injected with anti-APC antibodies or anti-Cdc20 antibodies. The distinction between cyclin A and cyclin B presumably depends on the recognition of their respective 'destruction boxes' which are not the same, and indeed not interchangeable. Cyclin A's destruction box is twice the length of cyclin B's, and point mutations that

1150 Virus-encoded proteinases of positive RNA viruses

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The strategy of encoding proteins in the form of polyproteins is extremely common (if not ubiquitous) in the positive stranded (mRNA sense) RNA viruses. Some viruses encode all of their proteins in a single open reading frame (ORF) whilst others encode multiple polyproteins. Virus polyproteins may contain proteinase domains which, either solely, or, in combination with host-cell enzymes, proteolytically process these precursors into mature products. Polyprotein processing broadly occurs via two types of cleavage; (i) extremely rapid, intramolecular (in *cis*), co-translational cleavages - generally referred-to as 'primary cleavages', or, (ii) 'secondary cleavages' - slower, intermolecular (in *trans*) events.

An important feature of the activity of some of these enzymes is the cleavage of specific cellular proteins which may serve to modify the host-cells macromolecular processes thereby assisting the replication of the virus within the infected cell.

Virus-encoded proteinases are molecular mimics of cellular enzymes. To date virus-encoded proteinases have been characterised using serine, cysteine and aspartate as active site nucleophiles, but some may be regarded as 'intermediate' in certain aspects: the 3C proteinase of picornaviruses have a serine proteinase-type fold but have a cysteine nucleophile.

The catalytic mechanisms, the role of these proteinases in regulating virus replication and their biotechnological applications will be discussed.

POSTERS:

CCS001 Production of proteolytic enzymes during development of the actinomycete, *Micromonospora echinospora*

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Members of the genus *Micromonospora* exhibit complex developmental cycles, differentiating both morphologically and physiologically. The physiology of the genus is poorly understood when compared with other actinomycete genera such as *Streptomyces*. It is believed that a greater understanding of *Micromonospora* physiology will lead to increased industrial potential for the genus. This particular study has focused on the production of proteolytic enzymes in the gentamicin producer *Micromonospora echinospora*. The production of extracellular proteolytic enzymes was studied in a complex liquid medium containing yeast extract and peptone. A rapid increase in extracellular proteolytic activity was observed prior to sporulation and increased throughout the stationary phase. Preliminary inhibitor and electrophoresis studies indicate the presence of multiple proteolytic enzymes, with their pH optima lying between pH 6.5 and 8.5.

This work may provide further evidence for the role of proteolysis in the developmental systems of actinomycetes.

CCS002 Cell-cycle regulated proteolysis in *Caulobacter crescentus*

ISABEL POTOCKA & M.R.K. ALLEY

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The Gram negative, aquatic bacterium *Caulobacter crescentus* has an unusual life-cycle in that it always divides asymmetrically to give rise to two distinct cell types, a stalked and a swarmer cell. The stalked cell is capable of DNA replication while the motile swarmer cell, which bears a single polar flagellum, is not competent for DNA replication until it differentiates into a stalked cell. The obligatory transition from a motile swarmer to a stalked cell is accompanied by the loss of the motility machinery including the single flagellum, which is replaced by a stalk at the same pole. We are interested in the turnover of the chemotactic machinery during the cell cycle. The protein level of McpA, one of the seventeen receptors for the chemosensory pathway, has already been shown to be modulated during the cell-cycle by targeted proteolysis in that it is degraded specifically during the swarmer to stalked cell transition. McpA is an integral membrane protein and thus extremely unstable once removed from the cytoplasmic membrane. As a consequence of that we have initiated a study to identify soluble components of the chemosensory apparatus that are degraded like McpA via a ClpX dependent pathway. The identification of cytoplasmically localised chemotaxis proteins will enable us to initiate biochemical studies on the cell-cycle control of degradation.

WEDNESDAY 12 APRIL 2000

1400 Drinking water: to drink or not to drink?

PIERRE PAYMENT

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After a century of so-called modern water treatments, what have been able to achieve and can we drink safely the water? The studies conducted by our group have clearly shown that when river water of marginal microbial quality is treated to the standards applied by the industry, one can still expect a significant level of waterborne illness in the population. We have suggested some plausible causes for the observed health effects: 1) failure to completely remove the pathogens, 2) failure to disinfect the pathogens, 3) recontamination of the water in the distribution system.

Some simple means can be used to reduce further the risks associated with drinking water and maintain tap water at a high level of safety. As a consumer, the risks associated with tap water are probably very small compared to other risks in life. As a scientist, the evaluation of these risks and their reduction remain an important objective. As a regulator and risk manager, having to balance all the risks and their socio-economic impact is a Gordian knot difficult to untangle.

A good part of the solution to water quality lies in education: basic sanitation can do a lot when people understand why they need to do it, scientists must learn to bring their discoveries to the public without being overly alarmist, schools and universities can teach the value of water and its protection, communication media must be able to support specialized journalists that will translate scientific jargon in layperson terms. All of this will enable every level of society to take the best steps to protect public health. This applies everywhere: from developing to highly industrialized countries there will be no better solution to accessible pure drinking water than exchange of information and education at every level.

1445 Modelling bathing water quality

NIGEL LIGHTFOOT

Group Director PHLS North

The implementation of Directive 76/160/EEC has led to significant improvements in bathing water quality throughout the European community. Currently the microbiological testing of bathing waters according to this Directive is carried out inter-mittently throughout the bathing season. The results of these tests are made available to the public some 48-72 hours after the sample is taken.

In environmental monitoring new developments have resulted in predictive approaches to the quality of urban air and of course weather forecasting. Such "apriori control systems" have existed for several years in industry where the setting up of quality assurance of processes has replaced the exclusive quality control of oral product testing.

Technical developments necessary to develop a warning system for bathing water exist, i.e. on-line measurements of effluents of waste water treatment plants, computerised modelling of water quality, bacterial diagnosis and die off models, geographical information systems applied to river basins and well established methods for weather forecasting.

There is now an opportunity to integrate these tools to provide bathing water forecasts so that the European citizen can be given all the information they need to carry out their own risk management of the potential health risks.

1600 Contemporary issues in aquatic microbial ecology, engineering and health

T. CURTIS

University of Newcastle upon Tyne

Most civil engineers and microbiologists admit that Environmental Engineering interventions have played a central role in Public Health in the developed and developing world. Yet some from the key institutions have yet to understand the implications of this observation in the education of Engineers and Microbiologists for the 21st century. The first generation of Environmental Engineers sought to eradicate miasma rather than microbes. Though conceptually flawed, their efforts had a tremendous impact (at considerable cost) on the creation of the supportable urban environment we know today. The second generation of Environmental Engineers sought a better understanding of the relationship between pathogens, engineering and disease. This is the faecal coliform generation. So called because, they and their microbiologist colleagues have been forced to use indicator organisms as surrogates of pathogens. The best of the second generation have exceeded their 19th century colleagues; engineering interventions that give more health to more people for less money. The worst have done the reverse. The best engineers have sought to achieve Brockian (after Thomas Brock) standards of microbial ecology with quantitative risk assessments. The lessons of the second generation are not well learnt. The engineering institutions have not encouraged this aspect of engineering education. Microbiologists have fared little better, vaccines remain more prestigious than sewage works and mathematical and quantitative aspects of microbial ecology have suffered during the molecular years. The same molecular revolution heralds a third pathogen generation who we will directly measure and engineer pathogens. The impact of this revolution will depend on whether we emulate the best or the rest.

1645 Environmental health and water - Back to basics!

S. PETRIE

Principal Environmental Health Officer / Richmondshire
Abstract not received

THURSDAY 13 APRIL 2000

0900 Educating engineers to fight infection in the 21st century

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Public health engineers are civil engineers specialising in water supply and sanitation. They need to be trained in 'sanitary microbiology' so that they have a basic understanding of how to design, operate and maintain microbiologically safe water supply systems and sanitation systems that contain excreted pathogens; how to design wastewater treatment plants to remove or kill pathogens; and how to implement wastewater reuse systems to produce microbiologically safe food.

Most water- and excreta-related infection occurs, and will continue to occur, in the developing world, and so public health engineers need to understand the environmental classifications of water- and excreta-related diseases; to know of the emerging and re-emerging water- and excreta-related pathogens; as well as, of course, the concepts of faecal

so as to prevent, to use Louis Pasteur's phrase, microbes having the last word.

0945 The unblamed culprits

ROGER HART

Consultant Environmental Officer tel: 0114-2367034

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As a self employed Consultant Environmental Health Officer my office parties are lonely events. However, working for myself I am spared the need to produce mission statements, value for money exercises performance indicators and all the other paraphernalia which "management" thinks up. I do, however, start many of my illustrated lectures with what would be deemed to be a mission statement. I explain to the audience what I do to:

- 1 Prevent Food Poisoning
- 2 Avoid Prosecution
- 3 Create a Due Diligence Defence
- 4 Educate & Train

Whilst I have seen results from my training in follow-up inspections where the staff worked, I have also become more aware of the part which others play who are not in the front line of food service or production. The safety of food behind the farm gate rests with the farmer but Vets will be involved and it will all be overseen by staff from M.A.F.F. A Government Department. M.P.'s become implicated as Edwina Currie found out over the Salmonella in egg issue. As soon as the food leaves the farm, hauliers become involved and continue to do so to & from the factories and to the consumer. Manufacturers of equipment have liabilities and responsibilities, and CE marking should protect us from any shortfalls. But does it? Who enforces it? Have they got the resources to enforce it? And, referring to enforcers, what about my own profession? Do they have the skills, the knowledge & the resources to advise manufacturers, caterers & consumers, and to enforce the law to prevent Food Poisoning? I have not even mentioned Television Cooks! Who are the unblamed culprits?

1100 Microbiological food safety - Getting the message across

A. KYRIAKIDES

J Sainsbury plc

Abstract not received

1145 A University for food and life – virtual, or a reality?

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Despite advances in recognising newly emerging food pathogens (STEC, prions, *C. jejuni* O:19), it is calculated that in the UK and USA 20-28% of the population suffer from food poisoning each year. Causes of food poisoning are frequently due to errors during food production and mishandling in the home. Most of these cases are avoidable if appropriate education of food handlers and the general public could be given. Routes of education through literature and training courses for food handlers and EHOs will be examined and their efficacy compared with the 'ABC approach' of behaviourists on how can one change a person's behaviour. Food safety education for school children through the National Curriculum and events such as the National Food safety week will be discussed.

Information sources and training aids on the Web (i.e. Food Safety Virtual University of the FSIS) will be compared with the East Midlands Development Agency proposed University for Food in the East Midlands. A fuller version of the talk will be available at **Error! Bookmark not defined.**

1230 Providing microbiologically safe food and drinking water: a global, vertically integrated approach to educating professionals and consumers

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Despite ample knowledge of mechanisms and control strategies, sporadic cases and outbreaks of enteric disease associated with established and emerging pathogens in food and water remain disturbingly common world-wide. Whilst resource, infrastructure and equipment deficits may offer a partial explanation in some circumstances, there remains a need to review education and incentivisation strategies for those involved in microbiological food and water safety which should extend to consumers.

A key element must be development of conversance with microbial ecology which underpins all departures from microbiologically safe food and drinking water resulting from failures to comply with the so-called G.S. Wilson Triad (Lancet 342; 1993: 1254). This demands longitudinally integrated management of contamination, colonisation and microbial metabolism. Education and training must also emphasise the value of adherence to meticulously standardised monitoring procedures for processes, controls and commodities.

We have previously advocated a new generation of food safety professionals who, despite differing educational backgrounds, will embrace public health, microbiological and environmental health skills (Int J. Env Health Res.7; 1997:137- 147). These principles have informed the creation of a new educational programme commencing in 2001. The MSc in the Public Health Microbiology of Food and Drinking Water is, a joint initiative between the Universities of Glasgow and Strathclyde, the Eijkman Foundation at the University of Utrecht and the Scottish Centre for Infection and Environmental Health but will evolve to embrace other countries and institutions.

WEDNESDAY 12 APRIL 2000

0910 Factors affecting growth and toxin production in foods – an overview

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The ability of foodborne pathogenic bacteria to bring about foodborne illness usually depends on growth, and in some cases also toxin production, within the food. Growth and toxin production are affected by cell history and by the environmental conditions during growth. Cell history will be reflected in the physiological state of the cells or spores. Factors such as the extent of sub-lethal damage and the difference between the previous and current environmental conditions (probably a combination of factors such as incubation temperature, pH and salt concentration) will influence the probability of growth and the lag phase of growth. Current environmental conditions will additionally influence the generation time of the organism, the final cell count and the quantity of toxin, if formed. The development of predictive models has led to much progress in quantifying the combined effects of environmental factors on growth and toxin production in foods. Further improvements in the ability to quantify factors affecting growth and toxin production will contribute to improved microbiological food safety.

0955 Foodborne botulism: it's just not home-canned foods

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Since 1980, thirty-six persons with foodborne botulism have been reported in Texas. Intoxication for three persons was associated with home-canned asparagus or chile peppers. Intoxication for other persons was associated with a beef meal stored in a Styrofoam container, aluminum foil-wrapped boiled potatoes stored overnight in water, or aluminum foil-wrapped baked potatoes stored on a kitchen counter. Most of the persons were intoxicated during a single outbreak which occurred in El Paso, Texas in April 1994. In this outbreak thirty persons were affected; four required mechanical ventilation; none died. All ill persons ate at the same Greek food restaurant. The attack rate among persons who ate a potato-based dip was 86% (19 of 22 persons) compared with 6% (11 of 176 persons) who did not eat the dip. The attack rate among persons who ate an eggplant-based dip was 67% (6 of 9 persons) compared with 13% (24 of 189 persons) among persons who did not. The same spoon was used to serve both dips. Botulism toxin type A was detected from patients and the two dips. Toxin formation resulted from holding aluminum foil-wrapped baked potatoes at room temperature, apparently over several days, before they were used in the dips.

1105 The microbiological quality of ready-to-use vegetable

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Unité de Technologie des Produits Végétaux, INRA, Site AgroParc, 84914 Avignon Cedex 9, France

Are considered here as "ready-to-use vegetables" products which have been processed to make them ready for consumption and which need refrigeration during storage. No

surface of raw vegetables. Microorganisms have therefore been a concern for the quality and the safety of ready-to-use vegetables. In spite of their rapid growth during storage, there are only few examples where microorganisms have been identified as the major agent of deterioration. As a consequence, the use of microbial counts as a criteria to assess quality may be contested in many cases. Occurrence of foodborne pathogens in ready-to-use vegetables have been described in various countries. It is strongly dependant on the environmental conditions of the primary production of vegetables and on the hygiene during processing. During storage, most foodborne pathogens survive or grow on ready-to-use vegetables, but growth rates and maximum populations are usually lower than those predicted from experiments in laboratory media. Availability of nutrients, presence of antimicrobial compounds, microbial competition, have been identified as factors limiting growth of foodborne pathogens.

1150 ¹⁴C-labelled plant cell walls: novel substrates for studying interactions between microorganisms food

C.J. BUCHANAN

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Microorganisms growing in plant foodstuffs have a variety of carbon sources available to them. A major carbon source is the plant cell wall, which once degraded will provide a variety of carbohydrates, amino acids, phenols and other small molecules such as methanol and acetate. Potential food spoilage organisms or food borne pathogens if they can break down plant cell walls will be able to access these nutrients and utilise them to sustain growth. Studies examining the metabolism of isolated polysaccharides or proteins provide valuable information but are limited since micro-organisms generally do not encounter isolated polysaccharides during growth on plant foodstuffs, but intact cell walls. Also, purified polysaccharides may be altered (e.g. loss of cross-links, loss of ester substituents) during the extraction process. To address this problem we have prepared plant cell walls from cultured plant cells in which specific carbon residues are radiolabelled in an otherwise "normal" cold cell wall. These cell wall substrates provide a simple means for studying the breakdown of plant cell wall components by food spoilage bacteria or food-borne pathogens in a realistic manner, and for studying potential interactions (e.g. cross-feeding of nutrients) between organisms during food spoilage.

1205 Natural toxins in shellfish (Offered paper)

L. SMITH

FRS Marine Lab, Aberdeen

Abstract not received

1220 use of live bacterial cultures for the control of food poisoning bacteria and fish pathogens

LONE GRAM, LILIAN NILSSON & BETTINA

SPANGGAARD

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Harmless live bacterial cultures offer promising solutions as inhibitory principles against some food borne human

paper discuss two examples related to seafood products of such usage.

The food-borne human pathogenic bacterium, *Listeria monocytogenes*, is a common contaminant of lightly preserved seafood products. As the organism is not inactivated during processing and as the bacterium may proliferate in the product, some level of risk of listeriosis is present. Many attempts have been made to develop new preservation techniques that can control *L. monocytogenes* and that do not alter the sensory properties of the product. *Carnobacterium* spp. are common members of the microflora of e.g. smoked fish, cause no adverse sensory effects, and are non-pathogenic. When present in the product in high numbers, they are able to completely inhibit growth of *L. monocytogenes* in cold-smoked fish. Part of this inhibition can be explained by production of bacteriocins.

Fish reared in mari- and aquaculture suffer, like all animals in intensive culture, from diseases. Although some may be controlled by vaccinations, antibiotics tend to be the method of choice for control, particularly in larval stages when the immune system is not developed. The use of antibiotics may lead to development of resistance in the microflora; this resistance may be transferred to human pathogenic bacteria present on the fish and subsequently result in un-treatable diseases in humans. Alternatives to disease control are needed, and addition of fluorescent pseudomonads or other beneficial bacteria to the rearing water has been shown to reduce reduce fish mortality, and can thus be used as an alternative to antibiotics for disease control.

1405 Distribution and survival of salmonella in farms, hatcheries and animal feed mills

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Salmonella infection in farm animals may result in clinical disease, especially in cattle, but more commonly herd or flock infections remain subclinical, representing a public health rather than animal health hazard.

When *Salmonella* enters a farm it commonly becomes widely distributed on the premises and may result in considerable local environmental contamination and infection of wildlife. Persistence on the farm results from either the long term presence of carrier animals or sequential infection of newly introduced animals following contact with a contaminated environment. The organism can survive in empty buildings for at least two years and individual infected mice can carry *Salmonella* for at least 8 months. On poultry farms all-in, all-out policies combined with effective terminal disinfection and rodent control can eliminate *Salmonella* from the premises and on cattle and sheep farms the development of herd immunity may result in the spontaneous clearance of *Salmonella*. Long term persistence of *Salmonella* is a greater problem on continuously occupied pig and egg production units.

Hatcheries may act as both reservoirs and amplifiers of *Salmonella*. Even in the absence of infected supply flocks *Salmonella* which has become endemic in the incubator ventilation system and other sites may lead to ongoing infection of day old chicks. Similarly, feed produced in a mill in which the pellet or mash coolers have been colonised by *Salmonella* may act as a source of infection for many years. The presentation will focus on dissemination and persistence of *Salmonella* in the environment using data derived from the author's investigations.

1450 Serotype-specific genes and surface antigens in the food-borne pathogen *Listeria monocytogenes*

(Offered paper)
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Abstract not received

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Farm animals are not born with zoonotic bacteria, they acquire them from their immediate surroundings on the farm. In the case of *Campylobacter*, farm animals are challenged with new strains throughout life on the farm. Pathogens can be isolated from faecally contaminated streams, water troughs, animal bedding, pasture, farmer's boots and in farm slurries and muck put to land.

Pathogens are brought onto farms by wild birds, such as starlings and sparrows, which roost in farm buildings, and gulls, corvines and oystercatchers, which forage on pasture, especially in windy weather, and in sewage sludge put to land.

Pathogens are spread round the farm when cows are brought in for milking, on foot wear, by dogs and cats, small birds, small mammals and flies. In spring and autumn, cattle and sheep shed more pathogens in their faeces (and muck and slurries) than at other times of the year. As a consequence the environmental loading with pathogens is greater at this time of the year.

Pathogens leave the farm in the intestines and on the coats of farm animals sent for slaughter, in milk, in slurries, muck and faeces washed into streams, in birds foraging on faecal material, and because of inappropriate behaviour of farm visitors.

1600 Filamentation of Salmonella cells: a common response to hostile conditions (Offered paper)

K.L. MATTICK¹, F. JØRGENSEN¹, J.D. LEGAN³, H.M. LAPPIN-SCOTT² & T.J. HUMPHREY¹

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In 1998, researchers in Exeter described the filamentation of *Salmonella* in response to chilling. Since then, filamentation of *Salmonella* has been observed under various other sub-optimal environmental conditions. The filaments produced in response to the different stresses appear identical by microscopy. They can be as long as 200 µm, are straight sided and have regularly spaced nucleoids. This suggests that filaments form as a result of inhibition of FtsZ, the protein required for the initiation of septation.

Filamentation occurs on the boundary between growth permitting and non-growth permitting conditions. At this boundary there appears to be a narrow range of environmental parameters that permit growth in terms of increase in biomass, but inhibit septation. Filaments are able to complete septation once the environmental conditions again permit growth. This has led to concern for food safety when conditions such as refrigeration are used to control pathogens including *Salmonella*. The organisms may continue to grow, forming filaments, and once heated or ingested many typical cells can quickly result through the completion of septation.

In this presentation possible mechanisms of inhibition of FtsZ, and possible benefits of filamentation for the cell will be discussed.

1615 Arcobacter characterisation; isolates from animals, poultry and humans

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Arcobacter butzleri and *A. cryaerophilus* were previously referred to as 'aerotolerant Campylobacter', but are now distinguished from the genera *Campylobacter*. However isolation methods are still being developed and hence their true occurrence in the food chain is probably underestimated. *Arcobacter* infections in animals are associated with abortions and enteritis whereas enteritis and occasionally septicaemia occur in humans. *Arcobacter* spp. have been detected in calves with diarrhoea and mastitis, as well as in clinically

Netherlands. *Arcobacter* spp., especially *A. butzleri*, like *C. jejuni* and *H. pylori*, colonise neonatal piglets and indicated an invasive potential. Serotypes 1 and 5 are primarily associated with human infection. The review will cover the growth characteristics of *A. butzleri* and *A. cryaerophilus* and toxin production. A full version will be available at: <http://science.ntu.ac.uk/external/fhc>.

1630 *Salmonella* and *Campylobacter* in the kitchen environment

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Cross-contamination is an important contributory factor in c.30% of *Salmonella* outbreaks. It is also important in outbreaks of *Campylobacter* and *Escherichia coli* O157 infection. The preparation of meals containing high risk foods, such as poultry meat and eggs, will cause the widespread dissemination of pathogens, such as *Salmonella* and *Campylobacter* spp., either by hand contact or by aerosols. When present on kitchen surfaces and utensils, *Salmonella* spp., in particular, are capable of prolonged survival, particularly in protective foods such as homogenised whole egg. The use of improved isolation methods for *Campylobacter* spp. is demonstrating that these bacteria, once thought to be particularly sensitive, are capable of surviving for over 24 hours on contaminated kitchen surfaces.

The control of foodborne disease involves intervention at all points on the food chain from farm to fork. It is important, however, that we do not over-emphasise the role of the consumer in the control process. It is difficult under normal domestic conditions to prevent the spread of *Salmonella* and *Campylobacter* spp. from chicken carcasses, for example, because contamination levels will be high and the design of most kitchens is not conducive to controlling the spread of bacteria. These and other matters will be discussed.

POSTERS:

EM01 Development of a transformation and gene reporter system for group II, non-proteolytic *Clostridium botulinum* type B

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Non-proteolytic, Group II strains of *Clostridium botulinum* are of particular concern to the food industry because of their ability to survive and grow in REPFEDs (refrigerated processed foods of extended durability). Their analysis would benefit from the availability of a gene transfer system. Initial attempts to transform ATCC 25765 with existing clostridial vectors were, however, prevented by a restriction barrier. Subsequent analysis showed that strain ATCC 25765 possesses a restriction endonuclease (*CboI*) and a methylase activity (*M.CboI*) which have the same specificity as *MspI* and *M.MspI*, respectively. *CboI* cleaves the palindrome 5'-CCGG-3' to generate a 3'-GC sticky end, whilst *M.CboI* specifically methylates the external C residue. Plasmids vectors prepared in an *E. coli* host expressing a methylase enzyme with equivalent specificity to *M.CboI* were subsequently shown to be capable of transforming ATCC 25765 at frequencies of up to 0.8×10^4 transformants per μg of DNA. Having developed an effective transformation procedure, we went on to construct reporter cassettes based on the *Thermanaerobacterium sulfurigenes lacZ* and the *Vibrio fischeri luxAB* genes. Using the former, we have obtained preliminary evidence that reporter genes may be used to evaluate the physiological factors that affect toxin production in the food environment

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Salmonella spp. are reported to cause ~30,000 cases of foodborne illness each year in England and Wales and as such are of great concern to the food industry. *Salmonella* cells have been reported to form filaments at a water activity (a_w) of approximately 0.95 or at a temperature of approximately 6 °C. In this study we have investigated whether the formation of filaments might be beneficial to the *Salmonella* cell in terms of stress tolerance, as this may allow cells to survive food processing and the low pH of the stomach. In addition, subsequent recovery of the filaments by septation can occur when conditions change, thus resulting in a larger number of cells being produced than appreciated by direct plating.

Filaments of *Salmonella enteritidis* strain LA5, formed over 1-5 weeks at low a_w (0.95) and/or low temperature (6 °C), were heat challenged for 80 minutes at 54 °C or acid challenged for 30 minutes at pH 2.8. Control cultures consisted of cells with the typical 'short' phenotype prepared in the same way and exposed to a similar stress for the same length of time. Samples were taken at pre-determined time intervals and plated onto blood agar (BA). Microscopy was also performed at pre-determined time intervals in order to monitor the change of cell morphology over time.

Filamentous cells consistently survived longer than small cells under stress conditions and filaments did not septate during heat or acid challenge. Such findings may highlight hazards associated with the storage of foods at low a_w or low temperature and may have considerable implications for our understanding of an infective dose for *Salmonella*.

EM03 Survival of *Salmonella* strains at high temperature and low water activity

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¹EMRG, University of Exeter, EX4 4PS, UK, ² PHLS Food Microbiology Research Unit, Exeter EX2 5AD, UK

Processing at high temperature and reducing water availability (a_w) are methods employed to reduce the risk of *Salmonella* food poisoning. However, it has been reported that *Salmonella* cells held at low a_w are more heat tolerant. In this study a number of serotypes of *Salmonella* were subjected to low a_w and high temperature conditions, to investigate strain variation. The strains included strains from food-poisoning outbreaks involving a food with a low a_w , strains isolated from cattle faeces and chicken carcasses, and *S. senftenberg* 775W known for its heat resistance.

Low a_w broths (a_w s of 0.65, 0.8 and 0.9) were prepared by the addition of high levels of glucose and fructose to TSB broth base, steaming, adjusting pH to 6.5 and then increasing a_w with TSB without added sugar. Stationary phase cells grown in TSB at 37 °C were inoculated into the low a_w broths and each was subjected to the temperatures 60 °C, 65 °C and 72 °C using a submerged heating coil in a Grant waterbath. At timed intervals samples were ejected from the heating coil, and cells were recovered on blood agar (BA) using the method of Miles and Misra. After 48hrs incubation at 37 °C the plates were counted and viable counts calculated.

The results indicated that *Salmonella* strains isolated from food-poisoning outbreaks involving a low a_w food were not necessarily more resistant to heat and low a_w than strains isolated from sporadic cases or the environment.

EM04 Effect of arsenic on antimony biomethylation by *Cryptococcus humicola*

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Biomethylation of metals and metalloids, such as antimony

antimony and particularly of microbial interactions with the metalloid. This is of particular concern when it is considered that antimony is used ubiquitously as a fire retardant and plasticiser.

We have shown previously that the yeast *Cryptococcus humicola*, a known biomethylator of arsenic, is capable of methylating antimony. The biomethylation pathway for antimony has not been established, but since the elements have similar physical, chemical and toxicological properties it is interesting to speculate on a common biomethylation pathway. When *C.humicola* cultures were supplied with both antimony III and arsenic, a reduction in the total amount of methylantimony species detected was observed. Increasing the ratio of arsenic to antimony further decreased the total amount of methylantimony in culture supernatants. This effect on antimony III biomethylation was evident when arsenic was supplied in either the III or V valency state, suggesting that competition was at an intracellular and not an uptake level. The presence of arsenic also resulted in a change in the relative proportions of di- and trimethylantimony present, with trimethylantimony becoming the predominant species. These data suggest that the enzymes involved in the arsenic methylation pathway are responsible for antimony biomethylation and that methylation of dimethylantimony to trimethylantimony is the rate-limiting step in the biomethylation pathway.

EM05 Detection of *Campylobacter jejuni* in seafood

KHALIL KERDAHI

US Food and Drug Administration, New York, USA

Abstract not received

EM06 Inorganic nutrient utilisation by "trained" *Pseudomonas putida* used in the bioremediation of agricultural soil polluted with crude petroleum

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Crude oil contamination of agricultural soil results in both physical and chemical environmental hazards. For example, polycyclic aromatic compounds present in crude oil can, when exposed to air and light, combine with substances present in the environment to form carcinogenic compounds (Sewell, 1975). Soil fertility problems can lead to death of plants and animals, and industrial and domestic water supplies may become contaminated. *Pseudomonas putida* is known to be capable of digesting crude oil in contaminated land, but little is known about the utilisation of inorganic nutrients during this bioremediation process.

Garden soil (2kg) was contaminated with 500g of Nigerian crude oil and inoculated with 250ml of molasses broth containing *Ps. putida* (PP) (9.30×10^6 cfu ml⁻¹) trained to selectively utilise 15% v/v crude oil using a method described by Nwachukwu *et al* (1999). The three replicates were kept at room temperature throughout the investigation. Control samples were set up for each replicate, which consisted of the same materials but without the PP inoculation (Amund and Igiri, 1990; Nwachukwu *et al.*, 1999). The mean change in population density of PP and total heterotrophs (TH) was calculated from plate counts of cultures inoculated onto microbiological media. Oil content of replicate soil samples was found using gravimetric methods and the inorganic nutrient contents (SO₄²⁻, PO₄³⁻, NO₃⁻, NO₂⁻, Cl⁻) of the soil were determined by computer-optimised ion chromatography (Dionex, DX-100 for anion analysis). Samples were taken weekly for 6 weeks.

In the test samples oil concentration and all the inorganic anions tested decreased more rapidly with proportional increases in the population densities of both PP and TH than

limiting. Inoculation of crude oil contaminated soil by PP is an effective way of bioremediation particularly in developing countries where sophisticated technology for oil pollution amelioration is not readily available. In bioremediation programmes inorganic nutrient concentration should be monitored closely, and adjusted to promote maximum biodegradation of oil.

REFERENCES: Amund, O.O and Igiri, C.U. (1990).

Biodegradation of petroleum hydrocarbons. World J Microbiol. Biotechnol. 6.225-262. / Nwachukwu, S.U., James, P. and Gurney, T.R. (1999) Training of *Pseudomonas*

aeruginosa as a polishing agent for cleaning environments polluted with hydrocarbons by step-up complex hydrocarbon utilization technique. *The International Journal of Environmental Education and Information*. 18(1) 53-66 /

Sewell, G.H. (1975) Environmental Quality Management .

Price-Hall Inc., Englewood Cliffe, New Jersey, 299pp

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EM07 Impacts of crude oil on the germination and growth of cress seeds (*Lepidium* sp) after bioremediation of agricultural soil polluted with crude petroleum oil using "trained" *Pseudomonas putida*

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The devastating impact of hydrocarbons on the normal morphology and physiology of several sensitive plants has been reported (Palaniswamy *et al*, 1995). However, bioremediation of agricultural land polluted with crude oil using microbes can help in regaining the land's fertility. This can be achieved in two ways: by enhancing the growth and activity of microbes already present at the site of pollution, and by adding more selected microbes to the pollution site (Ted and Udall, 1991). Following the bioremediation treatment, it is necessary to obtain some indication of the return of the land's fertility.

Garden soil (2kg) was contaminated with 500g of Nigerian crude oil and inoculated with 250ml of molasses broth containing *Pseudomonas putida* (PP) used as a bioremediation agent (9.30×10^6 cfu ml⁻¹) (Amund and Igiri, 1990). Two controls were set up. The first consisted of the same materials but without the PP inoculation. A second control comprised untreated garden soil. The experiment was replicated three times and kept at room temperature through out the 6 weeks investigation. All samples were examined for recovery from the impact of the crude oil by planting 100 cress (*Lepidium* sp) per sample. For 15 days after the first germination daily records were made of the number of germinated seedlings and their morphology.

At day 15 counts, there was 100% germination in the untreated control samples, the mean height of the seedlings was 75.83 %/- 2.60mm and all seedlings appeared to have growth morphologically normal. In the samples that had been treated with PP there was 98% germination and the seedlings had reached a height of 63.75 %/- 6.9mm again morphologically the seedlings appeared normal. However, in the samples treated with oil there was 38% germination and seedling heights of 42.33 %/- 8.50mm and some were stunted and had yellowing leaves. These observations indicate the adverse effects of crude oil. The data indicate that treatment with PP as a bioremediation agent does produce soil which is capable of growing larger and healthier plants than where bioremediation has not taken place.

REFERENCES: Amund, O.O and Igiri, C.U. (1990).

Biodegradation of petroleum hydrocarbons. World J Microbiol. Biotechnol. 6.225-262. / Palaniswamy, M., Gunamani, T., and Swaminathan, S. (1995). Effect of air

Microorganisms, Plants and Animals. Congress of the United States of America, Technology Assessment. Washington DC. 331pp

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EM08 Thermotolerance of *Salmonella typhimurium* and *salmonella senftenberg* after sublethal heat shock
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When bacteria are exposed to temperatures above their range for normal cell growth it can lead to a loss of cell viability. However when bacteria are shifted for a short period from lower to higher temperatures within or slightly above their normal growth range, synthesis of a specific set of proteins known as 'heat shock proteins' is induced. One consequence of this heat shock response is increased thermotolerance when the bacteria are exposed to the lethal effects of a subsequent shift to a higher temperature. The effect of heat shock response on food safety may be important as certain foods undergo thermal processing to ensure safety. This study was undertaken to determine the degree of acquired thermotolerance conferred on *Salmonella typhimurium* and *Salmonella senftenberg* by heat shocking at a range of temperatures and times. An investigation into the altered proteins produced following heat shock in comparison to non-heat shocked cells was examined by 2D SDS Gel Electrophoresis. Overall, an increase in thermotolerance was observed when exposed to an upshift in temperature in conjunction with a change in protein expression.

EM09 Assessment of microbial consortia for decolorisation of azo dyes under denitrifying conditions

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Various microorganisms reductively cleave azo dyes to the corresponding amines under anaerobic conditions. The aromatic amine products, which are suspect carcinogens and mutagens, are generally regarded as stable biotransformation products under these conditions.

The ability of three microbial consortia (garden topsoil, reed bed soil and canal sediment) to decolourise six azo dyes (Acid Blue 158, Acid Blue 113, Acid Red 73, Acid Yellow 99, Basic Red 18 and Direct Red 23) under denitrifying conditions was compared. Decolourisation was to >70% within 4-days, except for AY99 by the canal sediment (17%) and DR23 by the garden topsoil or reed bed soil (23-32%). The canal sediment transformed both AB158 and AR73 to aromatic amines stoichiometrically, whereas for the other dyes lower than expected levels of aromatic amines were found. Further processing of certain aromatic amines under anaerobic conditions would account for this. Matrix assisted laser desorption/ionization - mass spectrometry (MALDI-MS) was used for rapid identification of dye degradation products over the time-course of microbial decolourisation. Using glycerol as desorption matrix, several possible dye biotransformation products were detected, including p-nitroaniline, 4,4-diaminoazobenzene, chloroaniline and 1,4-diaminobenzene. These data illuminate the mechanisms of dye degradation by microbial consortia and have relevance to the design and operation of denitrification units for treatment of dyehouse effluent.

TUESDAY 11 APRIL 2000

0900 Water-borne disease - a global perspective

J WATKINS

CREH, Leeds, UK

Abstract not received

0940 Regulations and standards for the supply of potable water"

W.M. WAITE

Drinking Water Inspectorate, London

The author intends that the paper will consider the purpose and aim of microbiological regulations and standards, and comment on the extent to which current practices meet those aims. In doing this it will look critically at the origin and evolution of current UK standards, and their scientific basis and validity. It will also consider the practical consequences of current regulations, and whether they are a means to an end or can become an end in themselves. As part of this consideration the question of cost and benefit will be raised.

Having looked in some depth at the status quo the author will consider whether and how regulations and standards might develop in the future.

1050 Microbial risk assessment for treated drinking water

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This paper provides an overview of the analytical framework and analysis being conducted by USEPA for characterizing risks from pathogens in public water systems using surface water in the U.S. Data from the Information Collection Rule and Supplemental Surveys are used to characterize source water occurrence for Giardia, Cryptosporidium and viruses. Various interpretations of available source water occurrence data, treatment information, water consumption rates, dose response and immune response data are considered collectively to characterize possible levels of risk among utilities on a national basis. Even though significant uncertainties exist with many aspects of the analysis, EPA believes meaningful predictions can still be made for the purpose of supporting regulatory decisions. Preliminary risk estimates are given including key model assumptions and uncertainties in the analysis. Possible regulatory implications based on the risk analysis are also discussed.

1130 Balancing microbial and chemical risks of drinking water disinfection

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Balancing the positive (i.e. reduction of pathogen risks) and negative (i.e. increased chemical risks) effects of drinking water disinfection implies comparing different health outcomes with different probability and different severity.

To do so, a common basis is needed for both metrics.

Probability of adverse health effects

Comparing risks should be based on actual risk estimates

to assess exposure and dose-dependent effects, and to evaluate the impact of different treatment scenarios. Such models are based on numerous assumptions, which increases the complexity of using risk estimates in decision making.

Severity of adverse health effects

To quantify the health impact of different diseases, it is necessary to integrate the effects of mortality as well as morbidity. Mortality can be accounted for by the number of years of life lost (YLL), which is the difference between the actual age at death by a specific cause, and the life expectancy at that age. Morbidity can be considered to reduce the value of life during the period of overt disease and possibly of chronic sequelae. A severity weight, a factor between 0 and 1, accounts for the different level of impact that specific diseases will have on individual or population health. Thus, the loss of healthy life years due to morbidity (YLD – years lived with disability) is expressed as the time lived with disease, multiplied by the matching severity weight. The sum of YLL and YLD is the Disability Adjusted Life Years (DALYs), which quantifies the health impact of one or more diseases on a population.

Case study

The results of combining probabilistic risk assessment with the DALY concept is illustrated by a case study of ozonation of a hypothetical surface water supply. Only one microbiological and one chemical hazard are considered: *Cryptosporidium parvum*, leading to diarrhoeal disease in the immunocompetent and in the AIDS population, and bromate, leading to renal cell cancer. Under operating conditions, typical for Dutch surface-water treatment plants, the health impact of producing moderate amounts of bromate, is significantly outweighed by the health benefits of reducing the risk of cryptosporidiosis.

1330 The role of protozoa in potable water treatment

B.J. LLOYD

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Protozoa play a vital role in the removal of micro-particles, particularly bacteria and algae, in so-called 'biological' or slow sand filtration. This paper summarises results of *in vivo* surveys of the micro-fauna in full scale slow sand filters which demonstrate that the ciliate protozoa are one of the most constant features of the filter bed microbial community. The efficient performance of slow sand filters is highly dependent upon the rapid and sustained development of these protozoa throughout filter runs. Distinct protozoa ecotypes function differently in the removal of bacteria. Some species remove bacteria in suspension, others remove bacteria from the sand grain surface maintaining it receptive for the further removal of micro-particles by physico-chemical attachment mechanisms. Some protozoa species are carnivorous, consuming yet other protozoa and even multi-cellular members of the sand infauna! Dominant species of protozoa were cultivated *in vitro* and modelled in sand flow chambers to compare and validate their role and efficiency in filtration by comparison with sand alone. It was demonstrated that peritrich protozoa raised removal efficiency by 1 log¹⁰. The application of knowledge concerning the ecology and tolerance of the protozoa in optimising filter performance is discussed.

1410 Microbial quality of private water supplies in the UK

K. GIBBERD

1520 Microbiology of water distribution **CHERYL NORTON & MARK LECHEVALLIER**

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While modern drinking water treatment plants produce very high quality water, we know that a significant amount of bacteriological deterioration can occur within the distribution system. Research has shown that many factors influence changes in the microbial quality of distributed water, including treatment protocols, organic carbon content, disinfectant type and concentration, biofilm growth, corrosion levels and specific distribution system characteristics. Utility experiences have shown that coliform control is a multifaceted issue in many distribution systems, requiring unique solutions for each system. Pathogenic organisms such as *Cryptosporidium parvum* and *Mycobacterium avium* pose new challenges for potable water suppliers. *C. parvum* outbreaks have forced utilities to investigate its occurrence and control through treatment. Studies have shown that *M. avium* organisms survive in distribution system biofilms making human exposure a threat if sloughing or disruption of biofilms occur. New regulations requiring more stringent control of microbes in water with concurrent reduction of disinfection by-products continue to present new challenges for the water industry.

1600 Water treatment to prevent transmission of ***Cryptosporidium***

TOM HALL

WRc Processes, Swindon

Over the past ten years, outbreaks of cryptosporidiosis have been associated with water supplies, particularly in the UK and USA. This has drawn attention to the ability of water treatment to provide an adequate barrier for the pathogen. Disinfection processes normally used in water treatment are relatively ineffective for inactivating *Cryptosporidium*, and control of waterborne cryptosporidiosis has relied primarily on removal of oocysts by treatment processes. Conventional water treatment has been shown through pilot plant trials to be capable of achieving adequate removal of oocysts. However, to maximise oocyst removal, close attention needs to be given to particular aspects of treatment, and works operating practices need to reflect this. Recommendations for good practice have been provided by the Badenoch and Bouchier Reports, and by UK Water Industry Research.

The paper will review the performance of conventional treatment for oocyst removal, drawing attention to the areas of treatment plant design and operation which are important in minimising *Cryptosporidium* risk. This will include the tools and techniques which can be used to optimise treatment, some of which can also be used for the modular filtration systems and membranes being installed for treatment of groundwaters at risk from *Cryptosporidium*.

The new DWI *Cryptosporidium* Regulation does not take into account the viability of any oocysts found in water supplies, and therefore precludes inactivation as a control option in water treatment. In this context, the implications of recent developments in the USA on oocyst inactivation using ozone or UV irradiation will be reviewed.

1640 Statistical analyses and neural network simulations **of biological activated carbon filtration performances** **based on potable water microbial compositions**

MIKLAS SCHOLZ¹, NEBERAY K. MOHAMMED¹, SWATI MOHANTY² & MIKE J. SLATER³

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Both statistical and neural network analyses have been gaining importance in solving complex problems in the potable water industry. The purpose of this study was to predict the performance of Biological Activated Carbon (BAC) filters for contaminated river water (heavy metals and sewage). The identified micro-organisms include protozoa, algae (predominantly diatoms), rotifers, nematodes, bacteria and fungi. The parameters measured include pH, dissolved oxygen, biochemical oxygen demand and chemical oxygen demand (COD). Metal ions were absorbed by activated carbon and did not significantly inhibit microbiological growth. The interactions between environmental control variables, microbial composition on different filter media and microbial influence on the reduction of dissolved organic carbon were investigated with both parametric and non-parametric statistics (more suitable for aquatic microbiology). Empirical modelling was initially undertaken to enhance general understanding of the process and to identify saprobic indicator micro-organisms. Daily measurement data over a period of 105 days in spring have been used for training and validating the feed forward neural network architecture. The neural network simulation program has been successful in predicting the COD removal efficiency of a BAC filter. Strong correlations were computed. The average absolute deviation was 2.4% for the training and 15 % for the validation set.

WEDNESDAY 12 APRIL 2000

0900 Water re-use for potable supply

J. ROSE

University of South Florida, USA

Abstract not received

0940 Microbiological aspects of wastewater **treatment and reuse in developing countries**

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Wastewater treatment in developing countries needs to be low-cost, easy to operate and maintain and, in most cases, produce an effluent that can be safely used for crop irrigation and/or fish culture. The most appropriate treatment technology is usually either waste stabilization ponds (WSP) – one or more series of anaerobic, facultative and maturation ponds – or, in water-short areas, anaerobic ponds and wastewater storage and treatment reservoirs (WSTR); or a hybrid WSP-WSTR system.

WSP: anaerobic ponds are low-rate anaerobic reactors but nonetheless very efficient (over 70 percent BOD removal at 25°C). Facultative and maturation ponds are photosynthetic reactors with green algae providing the oxygen for bacterial BOD removal. The algae also create the optimal conditions for the rapid die-off of faecal bacteria.

WSTR permit the whole year's wastewater to be used for irrigation, rather than that just produced in the irrigation season, so enabling a greater area of land to be irrigated and more crops produced. The hybrid WSP-WSTR system also allows this to be done with half the year's wastewater treated and used for restricted irrigation and the other half for unrestricted irrigation.

Reuse quality guidelines. For restricted irrigation the WHO guideline is \neq 1 human intestinal nematode egg per litre of treated wastewater. For unrestricted irrigation there is the additional guideline of \neq 1000 faecal coliforms per 100 ml of treated wastewater. For wastewater-fed fishponds the guidelines are an absence of human trematode eggs and \neq 1000 faecal coliforms per 100 ml of fishpond water.

Properly designed WSP and WSTR can easily achieve these levels. For restricted irrigation and fish culture often only anaerobic and facultative ponds are necessary, whereas for unrestricted irrigation maturation ponds are needed.

G. HOWARD, S. PEDLEY, M. BARRETT, K. JOHAL, K. POWELL & R. TAYLOR
Robens Centre for Public and Environmental Health,
University of Surrey

The contamination of groundwater by pathogenic microorganisms is acknowledged as an important health risk worldwide. Epidemiological studies have shown that untreated groundwater is a significant source of waterborne disease in many countries, including the USA.

There are many possible sources of microbiological contamination to shallow groundwater. These include sanitation, animal husbandry, and other agricultural practices. In developed countries, particular problems include leaking sewers under urban areas, and contamination in rural areas from animal faeces and sewage-sludge spreading. In developing countries, microbiological contamination of groundwater is widespread and frequently arises from inadequate sanitation. Furthermore, poor sanitary maintenance of many groundwater supplies allows preferential flow paths to develop, causing rapid and gross contamination of sources following rainfall events.

Deep groundwater is generally assumed to be safe from microbiological contamination. Evidence is now emerging, however, to suggest that deep groundwater may also be vulnerable to contamination from more persistent microorganisms, particularly enteric viruses. The source of these organisms and the mechanisms by which they are transported to deep groundwaters are unknown, but the implications for public health are clear.

Understanding of the factors that affect the survival and transport of microorganisms in the subsurface is limited. This paper reviews current knowledge of the fate of pathogenic microorganisms in the subsurface. In particular, it considers microbiological loading from different sources, and the characteristics of potential pathogens from these sources that may influence their fate in the subsurface. In addition, the interaction between pathogens and the aquifer matrix, as it affects the transport and attenuation of pathogens, is discussed. The significance of these factors to the design of groundwater protection practices and approaches to water treatment is emphasised.

1130 Expanded bed groundwater treatment

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Groundwater normally has to be treated before it is suitable for potable use. For example by aeration, to supply oxygen; passage down through a sand filter, to filter out fine particulates and remove dissolved materials by microbial action; followed by a disinfection step, often using hypochlorite. However, many groundwaters contain trace quantities of ammonia, which reduces the anti-microbial efficiency of chlorination. We have investigated the potential of ammonia removal *via* oxidation to nitrate in a fluidized bed bioreactor.

A laboratory-scale expanded bed column (2.5 cm diameter by 50 cm height), containing coke colonised by a mixed population of nitrifying bacteria, to form biofilms approximately 0.5 mm thick (40-50 gdm⁻³ dry weight) was fed an artificial groundwater containing 1.9 mg/l NH₃-N. The bacteria were able to oxidise virtually all the ammonia to nitrate in a single pass, with a residence time of about 100 s through the bed (upward velocity approximately 0.5 cm s⁻¹). This equates to a throughput of 60 m³ groundwater per m³ expanded bed per hour.

POSTERS:

FB01 Influence of heavy metals and sewage on the microbial composition and purification performance of biological activated carbon filters

NEBERAY K. MOHAMMED & MIKLAS SCHOLZ

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Controlled biological regeneration of activated carbon filter material due to sorption and consequently metabolism of slowly biodegradable organic matter has recently been recognised by the potable water treatment industry. Regeneration leads to an extended bed life of biological activated carbon columns. The aim of this study was to solve filter clogging problems and improve the effectiveness and efficiency of filter media if traces of copper, lead and sewage are present in the inflow stream water. The interactions between environmental control variables, microbial composition on different filter media and microbial influence on the reduction of dissolved organic carbon were investigated. The new data sets were used to extend existing empirical models and to develop dynamic models, which were validated by experimental testing. All models were part of a predictive saprobic scheme used to optimise the overall filtration performance. It was found that low copper and lead concentrations do not inhibit microbial growth as much as expected due to media adsorption processes. In contrast, traces of sewage in the inflow water lead to a filter breakthrough of suspended solids and to an undesirable increase of the filter backwashing frequency as well as the occurrence of potential pathogenic bacteria including Coliforms (*E. Coli*).

FB02 Neural network simulation of biological activated carbon filtration efficiency based on potable water micro-organisms

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Neural networks have been gaining importance in solving complex problems in the potable water industry. The recent availability of neural computing toolboxes has made neural network application user-friendly. The purpose of this simulation study was to predict the performance of Granular Activated Carbon (GAC) filters, which have been transformed into Biological Activated Carbon (BAC) filters by formation of a viable biofilm during the bio-regeneration mode with untreated river water. By maintaining an ecological equilibrium between microorganisms, a BAC filter is more efficient in improving the quality of the filtrate than a GAC filter. The identified microorganisms include protozoa, algae, rotifers, nematodes, bacteria and fungi. The parameters measured include pH, dissolved oxygen, biochemical oxygen demand and chemical oxygen demand (COD). A critical requirement for the development of any neural network application is a large good quality data set. Therefore, daily measurement data over a period of three months have been used for training the neural network using the back-propagation method. The neural network has been successful in predicting the COD removal efficiency of a BAC filter (Empty bed contact time: 8 min.) by identifying and counting microbial organisms in water samples taken out of the liquid phase surrounding the carbon granules.

FB03 The effect of ozonation by-products upon the extracellular polysaccharide development of bacterial isolates recovered from potable water sand filters

SAMANTHA P. LAW, MAUREEN A.L. MELVIN & ANDREW J. LAMB

School of Applied Sciences, The Robert Gordon University, St Andrew Street, Aberdeen AB25 2HG

Slow sand filtration is a common method used to produce microbiologically safe drinking water. The unique feature of slow sand filters is the 'schmutzdecke', which acts as an interface for biofilm development and water purification. Water used from upland sources can become discoloured during heavy rainfall due to the presence of recalcitrant natural organic matter (NOM) which cannot be biodegraded. Ozone, a powerful oxidant, is used to remove discoloration prior to filtration. By-products of ozonation can act as substrates that feed directly into the TCA cycle. As more nutrients become available for growth, the bacterial biomass of the schmutzdecke increases, causing the sand filters to clog more rapidly, especially in the late autumn / early winter months. This clogging is thought to be due to the over production of extracellular polysaccharides (EPS) by the bacteria present in the schmutzdecke. Bacterial isolates, recovered from several filters, have been characterised with regard to their substrate utilisation. Using both biochemical analysis and scanning electron microscopy the EPS production of these species has been examined. Data from these investigations will be presented.

FB04 The detection and analysis of cyanobacterial toxins

GAIL M. ROBERTSON & LINDA A. LAWTON

The School of Applied Sciences, The Robert Gordon University, St Andrew Street, Aberdeen, AB25 1HG

Cyanobacteria (blue-green algae) are a group of photosynthetic prokaryotes. Toxins (cyanotoxins) produced by cyanobacteria exhibit extreme hepato and neurotoxic behaviour. Waters which are high in nitrogen and phosphorous are known as eutrophic and this can result in an increase in the growth of cyanobacteria. Cyanobacteria can form mass growths, or blooms, which accumulate in eutrophic waters many of which, include drinking water reservoirs. The most common toxins are the microcystins which are a class of cyclic heptapeptides produced by several genera of cyanobacteria including *Microcystis*, *Oscillatoria*, and *Anabaena*. Such toxins are released from the cyanobacteria when the cells are disrupted either as a result of cell death or when the cells become leaky with age. Microcystins are hepatotoxins and are known to lead to tumour promotion and haemorrhaging of the liver in both humans and animals.

There are a wide range of laboratory methods that can be employed to detect and identify cyanotoxins in water and cyanobacterial cells, including toxicity tests and bioassays as well as analytical methods including detection by HPLC-PDA. Currently there is no single method that can be applied which will allow satisfactory monitoring for all microcystins. Preliminary results indicate that detection of microcystins varies depending on the sample matrix and the extraction method.

FB05 Improved measurement of dissolved oxygen in bioprocess applications

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The availability of dissolved oxygen (DO) in a fermentation process is important for microbial production and growth. DO is typically monitored in bioprocess applications using a membrane-covered amperometric sensor. Significant errors in the displayed DO value can result when using an industrial DO transmitter to monitor bioprocesses, since most DO

difference in the partial pressure of oxygen (pO_2) and DO concentration. Unlike pO_2 , which is independent of composition, DO concentration, or oxygen solubility, varies with the composition of the solution. Since oxygen solubility in fermentation media is different from pure water, DO concentration expressed in parts per million (ppm) is incorrect for bioprocesses. The second problem arises when DO is displayed as percent saturation (%sat). If %sat is not compensated for pressure changes, ambiguous DO values will be displayed. For example, a pressure increase of 0.5 bar from ambient conditions in an air-saturated system will yield an error of ~50% in the displayed %sat value. Since the current output of the DO sensor is proportional to pO_2 , it is proposed that oxygen content be expressed as pO_2 to provide more consistent and reproducible DO measurements.

WEDNESDAY 12 APRIL 2000

0900 Fundamental issues in vaccine delivery

P. BEVERLEY

Jenner Institute, Compton

Abstract not received

0945 Balancing the immune system (the 'hygiene hypothesis')

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Recent increases in the incidence of allergies, inflammatory bowel disease, and certain autoimmune diseases are too rapid to be due to genetic change, so environmental factors must be sought. Epidemiological data are compatible with the view that the modern life style is depriving the developing immune system of educational inputs upon which, as a consequence of its evolutionary history, it has become absolutely dependent. Childhood virus infections fit neither the epidemiology nor the evolutionary considerations, whereas certain environmental saprophytic bacterial genera such as the mycobacteria are clearly a part of the evolutionary history of the immune system (as evidenced by the existence of specialised CD-1-restricted T cells that recognise mycobacterial glycolipids) and may function as natural regulators of Th1 activity, since in the absence of interferon gamma receptors they cause fatal infections. The evidence that contact with mycobacteria (including *M. tuberculosis* and BCG) can diminish the risk of allergies will be presented, as will their efficacy as treatments for allergy in animal models and in clinical trials in man.

Similar arguments apply to the increase in Th1-mediated autoimmune disease. The regulatory T cells that control such Th1-mediated inflammation can, paradoxically, be generated by appropriate Th1-inducing microbial preparations, and this may be particularly crucial in relation to heat shock proteins. Previous attempts to control autoimmunity by switching the response to Th2 may be misguided, and a consequence of a failure to distinguish between IL-4-expressing cells that regulate autoimmunity, and Th2 effector cells which can, in human and marmoset models, enhance it. Environmental bacteria may again be the required input.

1100 Getting live bacterial vaccines into people

D. LEWIS

St George's Hospital, London

Abstract not received

1145 Bacterial genetics as a route towards the delivery of mucosal vaccines and adjuvants

GORDON DOUGAN

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Studies on pathogens are beginning to elucidate the molecular mechanisms by which they colonise their hosts and induce immune responses. The identification of so called virulence-associated genes and their products will assist the development of genetically defined live vaccines, novel non-living vaccines and may even help in the generation of gene delivery systems for use in gene therapy. A major challenge will be to develop effective vaccines and vector systems that

vaccines and therapeutic agents will be developed. This presentation highlights some of the progress made in this area and discusses future prospects, particularly in respect to optimising delivery of antigens. Live vaccines and vectors can be based on defined mutants of enteric pathogens such as *Salmonella*, on viruses such as *Vaccinia* or even on parasites. Defined vector strains can be used for the delivery of heterologous antigens or even DNA, although stability of such strains is a major hurdle to be overcome before they can be used for practical purposes. I will discuss the progress towards the clinical evaluation of defined live vectors with special reference to *Salmonella*.

The development of mucosal vaccines based on non-living antigens is complicated by the fact that most pure antigens are poor mucosal immunogens. Indeed mucosal immunisation may induce a state of tolerance. Some enterotoxin molecules, including cholera-like enterotoxins, do possess mucosal immunomodulatory activities. Toxin molecules, which have evolved to interact with host cells, often showing specificity for particular cell types. Since the binding domains of toxins are often separate or can be separated from the regions responsible for cell damage, mutants can be constructed which bind to cells but are non-toxic. For example, site-directed mutagenesis can be used to create non-toxic derivatives of cholera-like enterotoxins that still bind to mucosal surfaces. These genetically engineered toxoids can be useful probes of the mucosal immune system and can be used to improve our understanding of how antigens are recognised and processed at the surfaces of the body. These studies may lead to a new generation of mucosal immunomodulators that will assist vaccine development.

1400 Antigenic changes affecting vaccine efficacy: detection and anticipation

BERNARD A. M. VAN DER ZEIJST

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Arms races between infectious agents and the immune system occur all the time. Typically, the infectious agent changes its surface antigen and becomes in this way 'invisible' for the immune response raised against the previous surface antigens. The speed of these events differs considerably. Sometimes it involves a battle within in the body of the patient. An initial immune response suppresses the infection, but after an antigenic change a burst of multiplication with accompanying disease occurs (e.g. sleeping sickness and *Borrelia hermsii* infections). In other instances it may take years, such as in the gradual replacement of capsule serotypes in *Streptococcus pneumoniae*. Also the mechanisms of antigenic variation may vary tremendously, from randomly occurring missense mutations to complicated DNA rearrangements. I will discuss two contrasting examples of antigenic variation in which vaccine efficacy has been affected or may become affected. The first topic is *Bordetella pertussis*, the causative agent of whooping cough. After the introduction of a whole cell vaccine, almost 50 years ago, mutations in virulence factors, such a toxin and pertactin, slowly arose. This has affected the efficacy of at least some vaccines. It may also lead to future problems with the new generation of acellular vaccines that consist of only 2 to 5 components. *Streptococcus pneumoniae*, the other example, is the causative agent of otitis media, pneumonia, bacteremia and meningitis. The recent licensing of a highly efficacious

conditions for such a scenario are present. Nasopharyngeal carriage of several serotypes in the same carrier is widespread. Also, exchange of genetic material between live bacteria, followed by homologous recombination, occurs. This has led in the past to emergence of antibiotic resistant serotypes. Limited data indicate that serotype replacement may have occurred during clinical trials. So far it is impossible to predict what will happen and how fast. We should monitor and try to model the events. If changes occur, the vaccine could be adapted by adding new serotypes (in a way that avoids unnecessary delays in licencing, e.g. using the influenza approach). But modelling also indicates alternative approaches, such as trying to reduce carriage or inhibiting genetic exchange. We will have to prepare several lines of defence, hoping that they will not be needed.

1445 Phage-displayed peptides: a new way to select immunogens for the development of anti-polysaccharide vaccines

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The major targets of the protective humoral immune response to Gram-negative bacteria are the capsular polysaccharides or the O-antigen carbohydrate moiety (O-Ag) of the lipopolysaccharide (LPS). Therefore, vaccination strategies have mainly focused on the development of polysaccharide-protein conjugates, which despite difficulties is the only viable strategy for human vaccination against carbohydrate antigens developed so far. Recent attempts have also considered anti-idiotypic vaccines based on mimicking the carbohydrate structure, but for several reasons their use in humans is still a matter of debate. In order to develop a new class of anti-polysaccharide vaccines based on the use of peptides, we and others have selected mimotopes, i.e. peptides mimicking protective saccharidic epitopes by screening of phage-displayed peptide libraries using protective monoclonal antibodies to the selected pathogen. Our model is *Shigella*, the Gram-negative bacterium responsible for bacillary dysentery. We are currently addressing several questions:

- How to efficiently deliver the peptides to the mucosal surfaces in order to test the ability of the mimotopes to elicit a protective antibody response?
- Is it possible to improve the immunogenicity of the mimotopes by improving the peptide sequences first selected?
- Which is the molecular mechanism of mimicking saccharidic epitopes by peptides?
- Is the affinity of the peptide-antibody interaction a parameter to discriminate between immunogenic and non immunogenic peptide mimics?

Preliminary data will be presented and the opportunity to assess a multivalent subunit vaccine strategy by combining in a single molecule, mimotopes of the protective carbohydrate epitopes corresponding to each of the relevant *Shigella* strains will be discussed.

1600 Microencapsulation and mucosal delivery of vaccine antigens, including DNA

H. OYA ALPAR

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There is an urgent need for pharmaceutically acceptable

microparticles show particular promise. Particulate polymeric carriers made from biodegradable synthetic polymers such as polyesters offer advantages in uniformity and flexibility. Earlier studies have demonstrated that microsphere-associated vaccine antigens and plasmid DNA can lead to significant immune responses, not only through parenteral delivery, but also through mucosal surfaces, especially through the nasal route, and that the responses induced can be optimised through formulation. These studies illustrated the importance of particle characteristics such as hydrophobicity, size and surface charge, as well as polymer type, molecular weight and crystallinity, on the modulation of immune response obtained. In these studies, the more hydrophobic particles gave higher immune responses than the less hydrophobic ones, positively charged ones better than uncharged ones, higher molecular weight and highly crystalline polymers better than lower molecular weight and less crystalline ones, and smaller particles better than larger ones. In addition, these responses may also be increased by the addition of bioadhesives. Recent publications from our group show the ability of mucosally administered microencapsulated recombinant subunit antigens derived from *Y. pestis*, to completely protect experimental animals from aerosolised challenge with virulent Plague causing bacteria. For the same microsphere preparation, when delivered intratracheally in a comparative study, which also investigated i.m. and i.n. routes of delivery, mucosal routes (both lung and nasal) gave equivalent or better immune responses than the parenteral route. Botulism toxins are amongst the most lethal substances known to science. We have now demonstrated that it is possible to fabricate microsphere containing a recombinant fusion of maltose binding protein and the 50 kDa carboxy-terminal binding domain of the Botulism toxin F heavy chain (MBP-FHc). SDS PAGE behaviour of the microencapsulated protein demonstrated a high degree of commonality with that of native antigen, implying that the microencapsulation process had not resulted in detrimental structural modification. Importantly, two appropriately timed intranasal administrations of microencapsulated MBP-FHc, was seen to engender solid protection following intraperitoneal challenge with more than 104 LD50s of pure Botulism toxin F. Again, in the context of clinically relevant antigens, I will also discuss the formulation of microparticulate systems which elicit strong humoral and cellular immunity after only a single mucosal or parenteral administration. We have also shown that, in addition to particle transference into mucosal inductive sites, such as NALT, we observed immunologically significant translocation of microparticulate material into systemic inductive sites, such as the spleen. These data will be discussed in the context of mucosal administration of microparticulate vaccine carrier systems.

1645 Cochleate delivery vehicles for the induction of mucosal and systemic immune responses

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Cochleate delivery vehicles are unique, scroll-like, multi-layered vaccine carrier and delivery formulations. The structural stability of cochleates and the protection of materials encochleated within them, allows for efficient delivery of proteins and polynucleotides in vivo by a variety of routes of administration, including mucosal (oral and intranasal), and parenteral (IM, ID and subcutaneous).

Cochleates as carriers for protein and peptide antigens effectively induce antibody and cell mediated immune responses. These include antibodies in the serum, saliva, gut, bronchial mucus and vaginal mucus. Differentiation of

been demonstrated. Protection from lethal and infectious dose challenge with viruses administered parenterally and mucosally has also been achieved.

DNA cochleates are highly effective immunogens by both parenteral and mucosal routes. DNA cochleates are more effective at stimulating antibody and cell mediated responses in HIV-1, Herpes Simplex Virus-type 1 (HSV-1), and HSV-2 systems. Results in these studies have included induction of antibody and cell mediated responses following oral administration, where naked DNA was ineffective. Protection from parenteral and mucosal lethal dose challenges has been demonstrated. Coadministration of encochleated IL-12 plasmids with HSV-2 gD plasmids supports stronger T helper 1 type responses and cytolytic T cell responses.

The safety and efficacy of DNA and protein cochleates has been confirmed and extended in several systems in other laboratories. Efficacy in gene therapy and drug delivery systems has also been demonstrated, and applications in these areas are being developed.

Acknowledgement: The collaborations of Michael Pride, Robert Natuk, Eric Mishkin, and John Eldridge of Wyeth Lederle Vaccines, and Massoud Daheshia, Nellie Kuklin, Sangjun Chun and Barry Rouse of the University of Tennessee, to this work are gratefully acknowledged.

POSTERS:

MI01 Investigation of plasmid DNA vaccines against coxsackievirus B3 induced myocarditis MARLEN AASA-CHAPMAN^{1,2}, DOMINIC WELLS² & HONGYI ZHANG¹

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Enteroviruses, in particular Coxsackievirus B3 (CVB3), are involved in over 50% of acute myocarditis and in approximately 25% of dilated cardiomyopathy cases. Prevention of CVB3 infection is therefore highly desirable. Attenuated variants of a cardiovirulent CVB3 strain (Nancy) generated in our laboratory have shown prophylactic effect in a murine model of CVB3 induced myocarditis. Attenuated live vaccines however are not without some risk. We are therefore considering DNA vaccination as an alternative approach to prevent human heart muscle disease.

Neutralising antibodies are known to play an important role in protection against enterovirus infection and the major neutralising epitopes have been mapped to exposed parts of CVB3 capsid proteins VP1 and VP2. The coding sequences for these two capsid proteins were incorporated in candidate DNA vaccine constructs. Expression of these proteins were confirmed by detection of mRNA transcripts using RT-PCR, and detection of proteins using immunostaining and western blotting of transfected cultured cells.

The immunogenic potential of the DNA vaccines was initially evaluated by direct intramuscular injection. Male SWR (H-2^d) and Balb/c (H-2^d) mice received two or three doses of plasmid DNA (each dose containing 50-200µg of DNA) and were then challenged with cardiovirulent wild type virus. Inoculations of DNA recombinant plasmid did not induce detectable levels of antibodies nor did they protect the mice from the development of myocarditis. We are now attempting to enhance the immunogenicity of the DNA vaccine candidates using an IL-10 encoding plasmid and alum.

MI02 Isolation, characterisation, cloning, sequencing, mutagenesis and expression of hyaluronidase from *Streptococcus suis* serotype 7

A G ALLEN S BOUTHO H LINDSAY D SEILLY &

Streptococcus suis serotype 2 causes porcine meningitis throughout the world, whereas in Scandinavia it is serotype 7 that is the primary aetiologic agent. Hyaluronidase (HAase), breaks down hyaluronic acid (HA) into constituent disaccharide units composed of 4-deoxy-L-threo-hex-4-enopyranosyluronic acid-[1-3]-N-acetyl-D-glucosamine (UA-[1-3]-GlcNAc) which absorbs at 232nm. Spectrophotometric analysis of the supernatants of several serotypes of *S. suis* revealed HAase activity in serotypes 3, 4, 7, 8 and 12. Purification of the activity from serotype 7 revealed a protein of approx. 120kDa, which upon N-terminal sequencing revealed the amino acid sequence NQLPSTETAS. The HAase gene (*hylA*) was cloned and sequenced from serotypes 2 and 7 revealing, in both, this amino acid sequence. The orf *hylA*₇ (3492bp) encodes a polypeptide of 1164aa. Analysis of *hylA*₂ revealed several point mutations and also a 21bp and a 3bp insertion at the 5' end of the gene. Moreover, a 2bp insertion, introducing a stop codon that would lead to the generation of a truncated orf, encoding the first 522aa of the full length HylA, was observed. Allelic exchange, introducing an erythromycin cassette into *hylA*₇ yielded a strain that no longer secretes HAase activity. Expression of HylA₇ under the control of the T7 promoter in pET21a (in BL21a) yielded active protein of the correct molecular weight. Furthermore testing of the serotypes for their ability to ferment HA revealed that only those serotypes that secreted HAase activity could ferment HA. This potentially could allow such serotypes to use *in vivo* derived HA as a carbon source to promote growth at sites of infection. Addition of exogenous purified HylA allowed the fermentation of HA by all serotypes, confirming that these serotypes can still ferment UA-[1-3]-GlcNAc.

MI03 Signature-tagged mutagenesis of *Burkholderia pseudomallei*

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Burkholderia pseudomallei occurs in tropical and subtropical climates and is the causative agent of melioidosis. Melioidosis used to be considered a relatively rare disease, but with improved diagnostic techniques and an increase in global travel, melioidosis is becoming more common and being isolated from more varied environments.

Signature-tagged mutagenesis has been used to study the genetic elements necessary for virulence in a wide range of pathogens. In this study signature-tagged mutagenesis was used to examine the virulence determinants of the pathogen *B. pseudomallei*. The technique relies on a pool of 96 plasmid DNAs each carrying a transposon that is tagged with a unique nucleotide tag. Recovery of this tag by polymerase chain reaction (PCR) allows identification of each transposon and hence each mutant. Integration of the transposon within the genome of *B. pseudomallei* was examined. Animals were inoculated with a pool of 96 transposon mutants. Organisms were recovered from infected animals and the tags present in the recovered pool of bacteria were compared to those present in the pool of bacteria used to inoculate the mice. By this method mutants putatively attenuated for virulence were identified. Selected mutants were then characterised *in vitro* and *in vivo*.

MI04 Immunogenicity and reactivity of three manufacturers' meningococcal serogroup C conjugate vaccines administered as a single dose in UK toddlers

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The successful introduction of meningococcal C conjugate (MCC) vaccines will depend on their ability to induce long-term immunity against meningococcal C disease. We assessed the ability of a single dose of MCC-diphtheria mutant toxoid conjugate vaccines (MCC-CRM197) or MCC-tetanus toxoid conjugate vaccine (MCC-TT) to prime for immunologic memory in toddlers. Children (n = 226) aged 12 to 18 months were randomised to receive a single dose of one of the three MCC vaccines at the same time as MMR immunisation. Evaluating antibody responses to meningococcal C polysaccharide vaccination 6 months later assessed induction of memory. Sera were collected before and 1 month after each immunisation and analysed for serogroup C-specific serum bactericidal antibody (SBA), IgG and antibody avidity. All MCC vaccines were well tolerated with minimal local and systemic reactions. MCC vaccines were immunogenic with 91 - 100% of children achieving SBA titres * 8 and 87.5 - 100% having a fourfold or greater rise in sba titre. SBA geometric mean titre (GMT) rose from <4 to 215 (95%CI 166-279). MCC-tetanus toxoid vaccine induced significantly higher SBA GMTs (p<0.001) and proportions with SBA * 8 (p=0.02) than the MCC-CRM197 vaccines. Though antibody levels decreased 6 months post-MCC, IgG antibody avidity rose significantly (p<0.001). SBA responses to polysaccharide vaccination confirmed the presence of immunologic memory, with SBA GMT increasing from 55.1 (95% CI 40-76) to 1977 (1535-2547) and 99% of subjects achieving SBA titres of * 8. A single dose of MCC vaccine in young children was well tolerated and immunogenic inducing high levels of anti-capsular and bactericidal antibody. MCC-TT vaccine is more immunogenic than MCC-CRM197 vaccines after a single dose. All vaccines primed for immunologic memory to meningococcal C polysaccharide and should induce long-term immunity to meningococcal C disease.

MI05 Licensed meningococcal A/C polysaccharide vaccine in adults induces immunological hyporesponsiveness

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Widespread use of meningococcal AC polysaccharide (MACP) vaccines has raised concerns about induction of hyporesponsiveness to meningococcal serogroup C polysaccharide. We investigated whether meningococcal C conjugate (MCC) vaccine overcomes any immunological refractoriness following MACP vaccine in adults. University students vaccinated 6 months previously with MACP vaccine, were randomised to receive MACP or MCC vaccine and antibody responses were compared to naïve students receiving MACP or MCC vaccine. In students primed with MACP vaccine, MCC vaccine induced significantly higher IgG and serum bactericidal antibody (SBA) levels than a second dose of MACP vaccine. Responses to a second dose of MACP vaccine were significantly lower than to the first dose. Previous MACP vaccine reduced SBA but not IgG responses to MCC vaccine compared to naïve students. This confirms that MACP vaccine induces immunological hyporesponsiveness to C polysaccharide in adults but this

MI06 Cross-reactivity of *Neisseria lactamica* with meningococcal antigens

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Acquisition of natural immunity to meningococcal disease during childhood is thought to result from nasopharyngeal colonisation by non-pathogenic *Neisseria* spp., and epidemiological evidence suggests that *N. lactamica* is the most important of these. As part of a project exploring the potential of *N. lactamica* to form the basis of a vaccine against meningococcal disease, particularly that caused by serogroup B strains, we have studied the degree of cross-reactivity between *N. lactamica* and *N. meningitidis*, using both human and murine sera.

Outer membrane proteins (OMPs) from *N. lactamica*, grown under iron limited and iron replete conditions, were Western blotted with convalescent sera from patients with meningococcal disease resulting from a range of *N. meningitidis* serogroups, serotypes and sero-subtypes. The results revealed a large number of cross-reacting protein bands which were distributed across a wide range of molecular weights, and found to be common to a number of sera. We have also shown that antibodies in the sera of mice immunised with *N. lactamica* killed whole cells or OMPs cross-react with a range of meningococcal serogroups, serotypes and sero-subtypes, using a whole-cell ELISA assay.

The cross-reactivity of *N. lactamica* with a range of *N. meningitidis* strains indicates that vaccines based on *N. lactamica* will provide broad protection against meningococcal disease.

MI07 Identification and characterization of peptides that mimic the epitope recognized by the *Neisseria meningitidis* immunotyping monoclonal antibody 9-2-13,7,9

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Although capsular polysaccharide based vaccines are proving beneficial in reducing the incidence of group A and C meningococcal disease world-wide, a suitable candidate for immunoprophylaxis against group B disease currently remains elusive; the primary reason is the poor immunogenicity of the group B capsular antigen in humans. While alternative targets such as lipooligosaccharide (LOS) have been implicated as putative vaccine candidates, the inherent endotoxic activity associated with these molecules raises serious concerns regarding their use. To address these issues we have undertaken studies to determine whether peptides that mimic epitopes displayed on *Neisseria meningitidis* LOS moieties can function as suitable immunogens for vaccination against group B disease. A variety of linear and conformationally restricted peptides have been identified by bio-panning peptide phage libraries with the high affinity meningococcal LOS3,7,9 immunotyping monoclonal antibody 9-2-L379. This immunotype is prevalent but not exclusive to the Group B meningococcus. Several peptide motifs were identified that bind to 9-2-L379 with similar affinities (KD=10-30 nM) as the native LOS antigen (KD=7nM). Evidence is presented to

peptide conformation without alterations in the primary sequence lead to a loss in mimicry. Immunisation trials are being undertaken to assess the potential of these peptides to elicit cross-reactive antibody responses to meningococcal LOS.

MI08 Antigenic variation of *Neisseria meningitidis* transferrin binding protein b (TbpB), a candidate vaccine against serogroup b disease

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This study, by large scale multiple sequence analysis has revealed that there is extensive heterogeneity of TbpB protein expressed in the by meningococcal in the UK population; an observation that has implications for the vaccine potential of the TbpB antigen. In the UK. *In vivo* expressed, surface exposed bacterial proteins are by their nature subject to immune selection. Therefore it is possible to predict that such proteins would be variable. The extent of variability would determine their suitability as a vaccine. It appears from this study that despite *tbpB* having been shown to be safe and immunogenic in humans and raise a bactericidal response in animals, that *tbpB* in the UK is so variable that it may not be possible to formulate a vaccine that effectively protects against disease from heterologous strains. The nucleotide sequences of the *tbpB* genes from 79 *N. meningitidis* strains isolated during the winter 1995/1996 and 18 strains representing well characterised known virulent clonal groups were determined and sequenced. SplitsTree analysis revealed that recombination and mutation events had occurred to separate the strains into 4 distinct but related groups. The first contained all 13 small *tbpB* genes (~1.7kb) representing one allele. Two ET37 complex strains also had identical small *tbpB* genes. The remaining groups contained diverse TbpBs with less than 47% homology to the smaller protein. Each of these groups comprised a number of related TbpBs, encoded by different alleles, from more than one meningococcal clone. This study provides evidence that diverse meningococcal *tbpB* genes have evolved through the combined effects of recombination as well as the accumulation of point mutations and that TbpB is probably subject to extensive immune selection. The effect of this antigenic diversity on the potential of TbpB as a vaccine component requires further immunological investigation.

MI09 The lysis of commercially available anti-cancer liposomes by *Clostridium perfringens* alpha-toxin

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Lipoburst is a putative method of site directed anti-cancer therapy, involving localised release of chemotherapeutic drugs at tumour cell surfaces. An anti-tumour cell surface antibody conjugated to a phospholipase C (PLC) from *Clostridium perfringens*, alpha-toxin, provide the means for lysis of anti-cancer drug loaded liposomes at tumour sites. Previously reported murine *in vivo* studies showed the inhibition of cell proliferation for two human cell lines by Lipoburst. In this study, we aimed to examine the enzyme kinetics behind the PLC-liposome interaction using two commercially available anti-cancer liposomes as model systems. The two liposomes investigated were daunorubicin loaded DaunoXomes and PEGylated doxorubicin loaded Caelyx. Both enzyme and liposome concentration affected the rate of release of the entrapped drug. Further analysis of

of activity occurred at high drug concentrations. PEGylation was also found to hinder the enzymes ability to lyse liposomes. The results generated will aid in the optimisation of the Lipoburst system as a method of site directed anti-cancer therapy.

MI10 Encapsulation of conjugate vaccines with *Bordetella pertussis* fimbriae as novel carrier proteins

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Haemophilus influenzae type b (Hib) capsular polysaccharide (polyribosylribitol phosphate, PRP) and *Neisseria meningitidis* group C capsular polysaccharide (MenCPs) are the active components of conjugate vaccines against bacterial meningitis. PRP and MenCPs are thymic-independent antigens and therefore are weak immunogens, which are not capable of inducing immunological memory. Conjugation of PRP and MenCPs to a protein carrier converts them to thymic dependency. In this study, novel vaccines with *Bordetella pertussis* fimbriae as carrier proteins have been assessed. Since most encapsulated bacteria colonise mucosal tissues, the induction of immune responses at these mucosal surfaces may limit or even prevent colonisation. Indeed, injectable use of the current Hib conjugate vaccines has been shown to reduce carriage of Hib in infants. However, immunisation with these vaccines via the oral route may be a practical alternative to conventional intra-muscular injection in order to optimise the mucosal response, but will necessitate radical changes in formulation. This can be achieved by microencapsulation in poly(lactide-co-glycolide)(PLG) microparticles. The methods used to characterise these high molecular weight novel conjugate vaccines and their encapsulation in PLGs will be described. *In vitro* release profiles and preliminary studies showing immune responses elicited by these formulations in animal models will be presented.

MI11 Investigation of the vancomycin resistance operon of *Enterococcus casseliflavus*

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Vancomycin-resistant enterococci are a rapidly increasing cause of nosocomial infections and are a prominent example of the growing problem of antibiotic resistance among a variety of pathogenic bacteria. *Enterococcus casseliflavus* possesses low-level, intrinsic, chromosomally encoded resistance to vancomycin and is of the VanC phenotype. Resistance is mediated by the production of altered peptidoglycan precursors terminating in D-serine, which have a lowered binding affinity for the antibiotic. Sequencing of the resistance operon has indicated the presence of two genes which exhibit a high degree of identity to VanXYc and VanT of *Enterococcus gallinarum*, 81% and 74% respectively. *E. casseliflavus* also demonstrates resistance to vancomycin that is inducible in its expression. The period of time required during induction before full resistance is expressed is unusually long. Analysis of the cell wall precursors produced during this time, by HPLC, indicated that growth was unable to resume until all the sensitive precursors terminating in D-alanine had been eliminated. Any sensitive precursor would be likely to sequester the lipid carrier necessary to translocate newly synthesised precursors across the cell membrane and it is therefore probable that growth is unable to resume fully until new lipid carrier has been synthesised.

MI12 Mutation of lipid A in *Neisseria gonorrhoeae*

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The ability of *Neisseria gonorrhoeae* to cause disease in humans is determined by its capacity to adhere and invade specific mucosal sites and to induce inflammation. Lipopolysaccharide (LPS) is a major constituent of the gonococcal outer membranes and is a well known mediator of inflammation. Lipid A, rather than the oligosaccharide components appears to be responsible for the inflammatory effect. We have attempted to investigate the role of lipid A in causing inflammation by cloning and disrupting the htrB (*waaM*) gene from the strain MS11 AroA. The cloned gene was inserted into plasmid pBR322, unique sites were created within the cloned fragment using reverse PCR. The gene fragment was then disrupted using an erythromycin gene cassette.

The resulting mutant bacteria harboured an altered LPS profile in comparison to the parent strain LPS. Using established *in vitro* stimulation assays, LPS of the parent and mutant were examined for their ability to induce cytokine production from human macrophages (U937) and human polymorphonuclear cells. Culture supernatants were harvested and assayed for cytokines by ELISA. The htrB mutant LPS induced a reduced level of cytokines in comparison to the parent LPS.

MI13 Characterisation of the specificity of the restriction/methylation system of *Clostridium difficile* and the development shuttle vectors

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Whilst *Clostridium difficile* produces two potent toxin molecules, other factors undoubtedly contribute to virulence. Ignorance of pathogenesis in this organism is exacerbated by the lack of effective gene systems to facilitate its analysis. Thus, there is currently no described procedure for transforming this important human pathogen. To develop such a system we have elected to generate a vector based on an endogenous *C. difficile* plasmid, and to rationally counter the effects of the organisms restriction systems through appropriate methylation of the plasmids employed. Accordingly, a plasmid has been isolated from *C. difficile* and its replication functions identified following the determination of its entire nucleotide sequence. Shuttle vectors based on this replicon have been shown to be capable of transforming, and to be stably maintained in, *Clostridium beijerinckii*. In parallel, we have determined that *C. difficile* produces three type II restriction/ modification systems. The restriction/methylation specificity of two of these has been determined and their effects countered through the production of plasmid vectors in an appropriate bacterial host. Characterisation of the third system is ongoing.

MI14 Virulence factor discovery in *Clostridium difficile* through modulation of transcription factor expression using anti-sense RNA

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The problems associated with *C. difficile*-associated disease management represents a significant burden on the resources of the Nations health service. This burden will increase with an ageing UK population. The rational development of therapeutic strategies to counter the threat of a particular bacterial pathogen requires a detailed knowledge of the

toxin B (270 kDa), represent the only known virulence factors. We have, therefore, devised a strategy which should enable the identification of virulence factors using anti-sense technology. A replication deficient recombinant expression vector (pMTL940C) has been constructed into which has been inserted a DNA fragment specifying an anti-sense RNA molecule directed against the *C. difficile* homologue of the *C. perfringens virR* gene (VirR is a transcription factor known to regulate the production of virulence factors in *C. perfringens*). This plasmid maybe delivered to the *C. difficile* genome, from a *Bacillus subtilis* donor, following its cointegration with Tn916. Inhibition of VirR production should lead to the down-regulation of those genes under its control, the products of which may be visualised on 2-D gels.

MI15 The role of lipid a in host-pathogen interactions in *Salmonella* infections of mice

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We are investigating the role of lipid A as an integral component of live, infectious *Salmonella* in the pathogenesis and immunity of invasive salmonellosis. Our work focuses on the molecular and cellular host responses in the early events leading to inflammation, focal lesion development and the induction of protective immunity, using lethal and sublethal infection models. We have constructed a *Salmonella* mutant with a deletion-insertion in the *waaN* gene and have shown that the mutant biosynthesises a pentaacylated lipid A molecule lacking a single fatty acyl chain. The *waaN* mutant was significantly less able to induce TNFa, IL-1b and iNOS responses *in vitro* and *in vivo*. In a lethal mouse infection model, the *waaN* mutant was highly attenuated, despite growing at the same rate as the wild type parent *Salmonella* in infected animals. This provided the first evidence that death in a lethal mouse typhoid infection is directly dependent on the toxicity of lipid A. We are extending these studies to understand the role of lipid A in the pathogenesis and immunity of invasive salmonellosis.

MI16 Persistence of sulphonamide resistance in *Escherichia coli* despite reduced usage

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This study investigated whether the reducing in use of sulphonamide antimicrobials in recent years had altered the prevalence and genetic nature of resistance among clinical isolates of *Escherichia coli*.

Collections of *E. coli*, from the Royal London Hospital in 1991 and 1999, were therefore compared. Phenotypic resistance to sulphamethoxazole was measured by determining the minimum inhibitory concentration (MIC); PCR was used to identify sulphonamide resistance genes.

There was no significant change in the prevalence of sulphonamide resistance between the two years: 39.5% of 362 isolates were resistant in 1991 and 45.2% of 378 isolates in 1999. This was during a period when sulphonamide use declined sharply, due to a switch in prescriptions from co-trimoxazole to trimethoprim.

Resistance to sulphonamides in *Enterobacteriaceae* can be determined by the *sulI* gene which is found in type I

42% of resistant isolates had *sull* and 66% had *sullII*; by 1999, 38% had *sull* whereas 79% had *sullII*. Thus, both the relative and the absolute frequencies of *sullII* had risen, despite low usage of streptomycin and the falling sulphonamide consumption. *Sull* was still present on integrons, *sullII* had moved to larger multi-resistance plasmids.

These data challenge the belief that the removal of clinical selection pressure should inevitably be followed by declining antibiotic resistance. The reasons for the conservation of this resistance are unclear; possible factors deserving further investigation include agricultural antibiotic usage and linkage of resistance determinants to other genes conferring a selective advantage.

MI17 Identification of cold induced genes in *Salmonella typhimurium*

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Salmonella is a major cause of food-borne illness in the UK and enters the food chain via contaminated carcasses of poultry and animals. Such carcasses are commonly stored in the cold for several days while being distributed to retail outlets and also, following purchase by the consumer. It is therefore of interest to ask how salmonella cells respond to such environments. We have previously reported the ability of a pathogenic isolate of *Salmonella typhimurium* to activate a multigenic stress response, the cold shock response, upon incubation at low temperature (Craig J.E. *et al.*, 1998, *Microbiology* **144**, 697-704). This seems to be a widespread, though not universal, bacterial response mechanism and is thought to adapt the cells for survival at low temperature.

Using a transposon which carries a bioluminescent reporter system, we have isolated a number of *S. typhimurium* mutants which exhibit increased light production at low temperature and have shown that one of the target genes encodes the cold shock protein CspB. We are currently characterising some of the other transposon mutants in order to identify the target genes and to gain insight into their physiological importance for adaptation to low temperature. We have approached this using a strategy of chromosomal digestion with *Sau3A*, followed by inverse PCR. This method complements that of proteomics and surpasses one of the shortcomings of 2D-gel analysis, by allowing cold regulated but non-translated RNA molecules to be identified. Using this approach we have identified a number of genetic loci which have not been shown previously to be cold shock regulated in bacteria, including some non-coding regions. A description of this strategy will be presented.

MI18 Optimisation of plga-microparticles for plasmid DNA encapsulation

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Oral administration of plasmid DNA (pDNA) encoding immunogens is an attractive modality for vaccination. We have previously developed a method for formulating microparticles composed of poly (D,L-lactic-co-glycolic acid) (PLGA) encapsulated pDNA with the use of the Silverson blender. The objective of this study was to investigate an alternative approach for microparticle preparation and to determine whether immune responses were elicited following oral administration. A marked improvement in total pDNA incorporation efficiency and percentage of supercoiled pDNA

methodology, a plasmid encoding a capsid protein of rotavirus type A, VP6 (pVP6), was administered orally to Balb/c mice. The results obtained demonstrated that one dose of PLGA-encapsulated pVP6 elicited rotavirus-specific serum antibody in the murine model. Antibody was not observed following administration of naked pVP6 by the same route.

MI19 Induction of systemic immune responses against measles virus using recombinant oral vaccines

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Immune responses were compared following administration of plasmid DNA (pMV64) or replication-deficient adenovirus vectors (RA68) containing a gene encoding the measles virus nucleocapsid protein under the control of the HCMV immediate-early promoter. Intramuscular or intraperitoneal inoculation of C3H/He mice with a single 150µg dose of naked DNA resulted in serum IgG responses specific to measles virus N protein in over 50 percent of the vaccinates. To determine the efficacy of oral delivery of plasmid DNA, pMV64 was first encapsulated in poly (D,L-lactic-co-glycolic acid) (PLGA) microparticles. Encapsulated DNA retained biological activity, as demonstrated by the transient expression of the N protein in human fibroblasts. Increasing titres of N-specific serum IgG antibodies were observed over a period of 3 months in 3 of 10 mice after oral administration of two 50µg doses of PLGA-encapsulated pMV64 DNA. A single oral dose failed to stimulate a response. In contrast, mice immunized in a single, oral dose of RA68, showed both an antigen-specific IgG response in serum and a corresponding MHC class I restricted cytotoxic T lymphocyte (CTL) response in spleens from 13 of 15 vaccinates. In summary, these results demonstrate the potential of orally-delivered transgenes to elicit systemic immune responses.

MI20 Pertussis toxin: characterising a vaccine component using physico-chemical techniques

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Pertussis toxin (PT) has been both chemically and genetically detoxified for use in whooping cough vaccines. However, the effects on protein molecular structure are poorly understood. In this study native and genetically detoxified PT (PT-9K/129G) treated with 0-0.5 % formaldehyde (FA), were analysed using combinations of: size exclusion chromatography (SEC), fluorescence, analytical ultracentrifugation (AUC), light scattering, and immunoblotting. Native PT and PT-9K/129G were shown by fluorescence and SEC to have no notable differences in secondary structure and subunit association. SEC profiles showed earlier eluting main peaks and additional species with increasing formaldehyde concentration. AUC, which analyses the protein in solution, and light scattering detection confirmed the presence of higher molecular weight species and aggregates in the formaldehyde treated samples. PT-9K/129G fluorescence displayed a red-shift with increasing FA concentration, indicative of protein unfolding. Fluorescence of the chromatography fractions revealed that the later eluting material was responsible for this red-shift.

S1-S5. It was indicated that there is cross-linking between close subunits with low FA. With increasing FA there is more random cross-linking of subunits and fewer antigens are recognised in the large aggregates. These techniques have proved to be effective in evaluating the molecular profile of PT used in vaccines.

MI21 *Salmonella* delivery of the *Yersinia pestis* V antigen as a candidate plague vaccine

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The V antigen of *Yersinia pestis*, the causative agent of plague, is a major protective antigen. A new plague vaccine consisting of purified V and F1 antigens of *Y. pestis* has been developed. We recently showed that V antigen, expressed as a fusion protein with F1 antigen in an *aroA* attenuated *Salmonella typhimurium* strain, provided significant protection against plague (1). Attenuated *S. typhimurium* strains have been well characterised in their use as carriers of heterologous antigens in the murine model to obtain insight into the optimal construction of live attenuated *Salmonella typhi* vaccines for use in man. They are attractive for use as carriers since they can be administered orally and are capable of eliciting both systemic and mucosal immune responses, which may be important in protecting against bubonic and pneumonic plague. In these studies, the potential of *Salmonella* delivery of V antigen as a vaccine for plague is evaluated.

(1) Leary SEC, et al. *Microb Pathog* 1997; 23: 167-179.

MI22 Expression of recombinant heat shock protein 60 from *Francisella tularensis* and evaluation of its ability to induce protective immunity

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Francisella tularensis is the causative agent of tularemia a disease of man and animals. Currently an attenuated strain, *F. tularensis* LVS, is used as a vaccine in humans although the nature of the protection has not been ascertained and the protective antigen/s have not been identified. *F. tularensis* LVS however, remains virulent in mice and is thus the model of choice for vaccine studies. In this study the gene encoding HSP60 in *F. tularensis* LVS was PCR amplified and cloned in frame into the expression vectors pGEX-5X-2 and pRSETA. Recombinant proteins expressed in pGEX-5X-2 were fused to an N-terminal glutathione-S-transferase protein and proteins expressed from pRSETA were expressed with six histidine N-terminal residues. The resultant fusion proteins were expressed in suitable *E. coli* host strains and purified using affinity chromatography. Immunisation studies to assess the protective immunity afforded by these recombinant proteins against *F. tularensis* have been evaluated in Balb/c mice.

MI23 The capsular polysaccharide locus of *Streptococcus pneumoniae* serotype 6B

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Streptococcus pneumoniae is an important human pathogen. It is the primary causative agent of community-acquired pneumonia, and a major cause of meningitis and otitis media. *S. pneumoniae* ("the pneumococcus") possesses a polysaccharide capsule that is essential for virulence. Ninety antigenically different pneumococcal polysaccharide

The capsular polysaccharide locus (*cps*) of *S. pneumoniae* serotype 6B has been sequenced using a combination of cloning, long PCR, and using primers designed from the DNA sequences of conserved genes of other serotypes.

The DNA sequence of the serotype 6B locus is 15.1kb in size and consists of 15 ORFs. The first four ORFs (*cps6bA-cps6bD*) and the eleventh (*cps6bJ*) exhibit moderate to higher DNA homology with ORFs from *cps* loci of other pneumococcal serotypes and are postulated to be responsible for common functions. The tenth ORF (*cps6bI*) has homology at the amino acid level to other pneumococcal polysaccharide polymerases. Five ORFs (*cps6bE, cps6bL, cps6bM, cps6bN* and *cps6bO*) have high DNA homology to other serotypes that share structural elements (glucose and rhamnose). The remaining four ORFs are different to those from previously characterised *cps* loci. A putative pathway for the biosynthesis of the 6B capsule is proposed.

MI24 Molecular characterisation of interactions between *Salmonella typhimurium* mutants and murine macrophages

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Salmonella spp are widely regarded as facultative intracellular pathogens. Indeed, the ability of some *Salmonella* spp to cause systemic infections may be linked to survival for extended periods of time in macrophages and other phagocytic cells. We have been employing *in vitro* and *in vivo* approaches to investigate the molecular basis of the interaction of *Salmonella* with macrophages. The *in vitro* work has focused on cultured J774A.1 murine macrophages whereas the *in vivo* work utilises cells derived from different murine strains harbouring specific gene knock outs. Work has focused on derivatives of the *S.typhimurium* strain HWSH harbouring mutations in different genes known to be involved in intracellular survival. Detection of the *S.typhimurium in vitro* and *in vivo* is facilitated by the use of GFP reporters whose expression is dependent on the stationary phase activated promoters *spv* and *dps*. Preliminary data suggests these promoters are activated within 30 minutes of *Salmonella* entry.

MI25 Characterising monoclonal antibodies that protect mice against *Yersinia pestis*

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V antigen (Vag) is a 37 kDa protein produced by *Yersinia pestis*, the causative agent of plague. Vag has been reported to play two roles in type III secretion, both as an intracellular regulator of protein expression and as a surface-located protein. Vag is highly immunogenic and, along with F1 antigen, forms the basis of a next-generation plague vaccine. We previously identified a monoclonal antibody (mAb 7.3) that protected mice in a model for bubonic plague. Here we report the identification and characterisation of a second protective antibody (mAb 29.3). Western blotting against truncated forms of Vag indicates mAbs 7.3 and 29.3 recognise conformational epitopes that co-localise to aa 135 to 275 of Vag. However, the antibodies can be differentiated by their recognition of Vag from other pathogenic *Yersinia*. In addition, competitive ELISAs demonstrated that mAb 29.3 recognises *Y. pestis* Vag mutated at aa 255 more strongly than mAb 7.3. This suggests the presence of at least two protective epitopes within the central region of Vag.

MI26 Evaluation of a sub-unit vaccine against the type F neurotoxin of *Clostridium botulinum*

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toxin exist (A to G) and exposure to microgram quantities of the toxins leads to a flaccid paralysis and death. Although the most likely source of exposure to the toxin is through spoiled food there is potential for the toxin to be used as a biological weapon. We are investigating the feasibility of producing sub-unit vaccines against the botulinum toxins which could be used to protect military personnel from such an attack. In this study we evaluate the effectiveness of a genetically engineered sub-unit vaccine against type F botulinum toxin. A synthetic gene encoding the Hc (binding) domain of *C. botulinum* neurotoxin F (Fhc) was expressed in *E. coli* as a maltose binding protein fusion (MBP-Fhc). The cleavage product, Fhc, was evaluated for its ability to protect mice against toxin challenge. Vaccination of Balb/c mice with Fhc resulted in protection against 10^4 LD₅₀ doses of botulinum toxin F when administered by the intraperitoneal route. The minimum protective dose of vaccine (single and multiple vaccinations) and the time taken to achieve protection was also investigated. Protection was achieved within 10 days following a single 1 µg dose of the Fhc vaccine. Protection against an aerosol challenge of toxin was also demonstrated.

MI27 *In vivo* characterisation of quorum sensing-defective mutants of *Yersinia pestis*

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Quorum sensing is the ability of bacteria to regulate gene expression in a cell density-dependent manner and is mediated by signal molecules such as the *N*-acylhomoserine lactones (AHLs). Two regulatory genes, homologues of the *luxI* and *luxR* genes of the marine symbiont *Photobacterium fischeri*, encoding an AHL synthase and a transcriptional activator protein respectively, are involved in quorum sensing in a variety of Gram-negative bacteria. Such systems are known to play a role in the regulation of virulence in several species and multiple systems have recently been identified in a number of pathogens including *Pseudomonas aeruginosa* and *Yersinia pseudotuberculosis*. The causative agent of bubonic plague, *Yersinia pestis*, expresses a complex array of virulence factors, the regulation of which is poorly understood. Previously, we identified two *luxRI*-type quorum sensing systems in *Y. pestis*, sequenced the regulatory genes, designated *ypeRI* and *yepRI*, and characterised the AHLs produced by each of the AHL synthase genes in *Escherichia coli*. In this study, we describe the *in vivo* characterisation of three quorum sensing-defective mutants using median lethal dose and competition experiments in the mouse model and flea blockage experiments in the flea model. We also compare the protein profiles of each mutant with wild type *Y. pestis* using two dimensional SDS-PAGE.

MI28 Physiology and pathogenicity of *Mycobacterium tuberculosis* grown under defined conditions

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New vaccines and therapeutics are required for more effective control and prevention of tuberculosis. These strategies depend on improving our understanding of mycobacterial pathogenesis. We have developed a chemically defined culture medium and established growth of *Mycobacterium*

macrophage invasion assays and by aerosol challenge of guinea pigs. Steady state growth was achieved under aerobic conditions (dissolved oxygen tension of 50 % air saturation) at a mean generation time of 24 h. Cultures reached steady state cell densities of 5×10^8 cfu ml⁻¹ and were dispersed suspensions of short rods 2 to 3 µm long surrounded by a fine layer of capsular material. The Tween 80 content of the medium was optimised at 0.2 % (w/v) to facilitate dispersed growth. Cells grown under controlled conditions were pathogenic, and capable of causing pulmonary disease in guinea pigs. Aerobic chemostat cells were significantly more invasive for mouse macrophages than cells grown in conventional aerobic batch culture and on Middlebrook agar, demonstrating that the growth environment can induce a more invasive phenotype. *This work was supported by the Department of Health, UK.*

MI29 A single-administration toxoid vaccine based on biodegradable microspheres

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Polyester microspheres (MS) have shown potential for single injection vaccines. However, lack of boosting effects in animals has been observed and generally ascribed to insufficient long term antigen stability within MS. This study examined the effect of co-encapsulated putative stabilising additives on diphtheria toxoids (Dtxd) in different poly(lactide) (PLA) and poly(lactide-co-glycolide) (PLGA) polymers. We investigated the influence of MS size and polymer hydrophobicity of commercial and novel modified polymers on toxoid entrapment, antigen release and immunogenicity in guinea pigs.

The co-encapsulated stabilisers strongly influenced the entrapment of Dtxd in MS. Whereas albumin and trehalose lowered Dtxd entrapment in spray-dried MS, albumin increased it in coacervated MS. Further, entrapment decreased as a function of polymer hydrophobicity in spray-dried MS. Modified PLAs, carrying stearyl moieties, showed very promising entrapment. Here, albumin was a prerequisite, as other formulations produced strong toxoid precipitation.

MS-based toxoid vaccines were compared with alum-based vaccine in respect to antibody responses, monitored up to 40 weeks, determined by ELISA and toxin neutralisation assays. Ab-responses to Dtxd were significant for MS based on hydrophilic, fast-releasing polymers (PLGA 50:50) and were comparable to the responses for alum adsorbed vaccine. Unexpectedly, large (20-50 µm) and small (1-5 µm) MS gave comparable primary Ab-responses. None or very low Ab-responses were determined after immunisation with MS of hydrophobic polymers. All MS formulations designed to provide the priming and one boosting dose elicited protective Ab-responses throughout the 40 week study confirming the feasibility of MS vaccines to induce long-lasting protective response after a single dose. Long-term immunogenicity of MS vaccines for mixtures of toxoids is under investigation.

MI30 Heterologous expression of LcrV and LcrG: proteins critical for the type III secretion system of *Yersinia pestis*

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LcrV and LcrG are key proteins in the type III secretion system of *Yersinia*. LcrV is a secreted protein, required for the translocation of Yop effectors into the mammalian cell. It is also regulatory, and enhances Yop expression and secretion

LcrG is believed to be an intracellular "gate", blocking type III secretion under non-inductive conditions.

The LcrV and LcrG interaction may be facilitated by coiled-coil domains. These are important for several protein interactions in type III secretion systems. Indeed predicted coiled coil domains are present in both LcrV and LcrG. Moreover a deletion within this region in LcrV functionally inactivates the protein.

The aim of this work is to probe the importance of such domains in the interaction of LcrV with LcrG. Initial studies have investigated affinity-tagged and unfused protein expression systems for LcrV and LcrG. An ELISA protocol has been developed to demonstrate the interaction between LcrV and LcrG. Furthermore, LcrV proteins mutant at Leu residues shown to be essential for coiled-coil interactions have also been generated.

Using these recombinant proteins it may therefore be possible to determine amino acids important in the functional interaction of LcrV with LcrG within *Yersinia* type III secretion.

MI31 Development of novel isothermal amplification assays for the detection of bacterial nucleic acids

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Two novel isothermal nucleic acid amplification assays are being developed for the detection of specific DNA or RNA sequences from infectious disease agents: the first is Signal Mediated Amplification of RNA Technology (SMART) and the second, Split Promoter Amplification Reaction (SPAR).

Both assays employ probes containing sequences which are partly complementary to the target nucleic acid and partly to each other, forming a unique structure with the target called a three way junction. The assays require no thermal cycling, or reverse transcriptase in the case of RNA detection, and when fully developed will be carried out in a single tube. Target dependent signal is generated by the activation of a T7 RNA polymerase promoter, which is possible only in the presence of the target nucleic acid. The RNA signal generated in both assays from any target is always the *same sequence, hence the end detection probes and conditions are identical.*

We have used a number of model systems to test the efficacy of the assays on complex genomic material from viruses and bacteria. We present results for the detection of specific sequences in *Escherichia coli*.

MI32 Anti-microbial mechanisms in *Salmonella* infections

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Salmonellae infect phagocytic cells *in vitro* and *in vivo*. In the mouse typhoid model, the suppression of bacterial growth in the tissues requires granuloma formation and macrophage activation with the contribution of cytokines such as TNF α , IFN γ , IL12 and IL18. Recent studies using gene-targeted mice deficient in the NADPH oxidase-dependent oxidative burst (gp91 $phox^{-/-}$) and/or in the inducible nitric oxide synthase (NOS2 $^{-/-}$) have shown that both reactive oxygen intermediates (ROI) and reactive nitrogen intermediates (RNI) are required for host resistance to *Salmonella*. However, the temporal contribution of ROI and RNI to host resistance was very different. Studies in elicited peritoneal macrophages showed that the ROI dependent killing is confined to the first

results. In fact, higher bacterial numbers were present in the spleens and livers of gp91 $phox^{-/-}$ mice as compared to C57BL/6 controls from day 1 of the infection, and the gene targeted mice died within 4 days. Conversely, differences in

bacterial numbers were detected in the tissues of NOS2 $^{-/-}$ mice and C57BL/6 mice only after the first week of infection. The results show that both the NADPH oxidase-dependent oxidative burst and NOS2-mediated production of RNI are required for host resistance to *Salmonella* and operate at different stages of the infection.

MI33 Evaluation of antibodies and antibody fragments for use as an antitoxin to *Clostridium botulinum* neurotoxins

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Exposure to *Clostridium botulinum* neurotoxin causes flaccid paralysis and death unless prompt treatment is given. In classical botulism the toxin is ingested in spoiled food, although *C. botulinum* can colonize the gut of young children, causing infant botulism. There is also the potential for exposure to toxin by inhalation if used as a biological weapon. At present the only available treatment for intoxication is the immediate administration of an equine antitoxin. Supplies of the equine antitoxin are limited, and have the disadvantage of a relatively high occurrence of side effects (estimated to occur in around 20% of cases). F(ab')₂, Fab' or Fab antibody fragments may be more useful as botulinum antitoxins, as the despeciated fragments should show a reduction in side effects, due to the loss of the complement fixing F_C region. It is likely that fragmentation of an antitoxin will change its pharmacodynamics and efficacy. Smaller antibody fragments are expected to have a larger volume of distribution, which may widen the therapeutic window, increasing the efficacy of the antitoxin. However, antibody fragments may have an altered affinity for the toxin, and the loss of the F_C region may reduce the efficiency of toxin elimination, decreasing the efficacy of the antitoxin. We are currently investigating the pharmacodynamics and efficacy of IgG and antibody fragments of a botulinum antitoxin. This will enable us to select the most appropriate fragment for the manufacture of an improved antitoxin suitable for human use.

MI34 Multivalent vaccination by selective antigen delivery to macrophage cells

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Live attenuated *Salmonella* strains can be used as vehicles for displaying foreign antigens and provide a route for eliciting simultaneous protection against *Salmonella* and other pathogens. A number of promoters of *Salmonella typhimurium* (e.g. *pagC* and *ahpC*) are known to be activated during macrophage interaction (Dunstan *et al.* (1999) *Infect. and Immun.* 67:5133-5141; Francis K.P and M.P. Gallagher (1993) *Infect. and Immun.* 61:3208-3217; Taylor P.D., *et al.* (1998) *Infect. and Immun.* 66:3208-3217 and Valdivia *et al.* (1996) *Gene* 173:47-52). Such promoters are particularly attractive as a means for regulating antigen expression because they are activated in response to interaction with cells which play a central role in microbial destruction, antigen presentation and stimulation of the immune response. We have utilised these features for the plasmid-mediated expression *in situ* of a number of foreign bacterial and protozoan antigens from pathogens, and the role of these antigens in eliciting protective immunity is being addressed as part of this study. The antigenic regions under study are

have been chosen because of their ability to be recognised as part of the cell-mediated arm of the immune system during the course of infection of BALB/c mice by the native organism. Each region is also long enough to potentially encode B cell epitopes.

A plasmid system has been designed which is based on complementing an auxotrophy (purine requirement) in order to facilitate its persistence within the host (i.e. the *salmonella* cells require purine to persist within the host and so will only survive if they maintain and express the plasmid borne-gene *pur A*). In the present study the gene fragments encoding each of the antigens of interest have been cloned sequentially into the plasmid, downstream of the macrophage-induced promoter, thereby forming a plasmid which encodes a large polyvalent fusion protein. The effectiveness of these promoters for antigen delivery and immunisation via an attenuated *Salmonella* vehicle is currently being assessed using a BALB/c mouse model. Whether this strategy elicits a T_H-1 or T_H-2 type response is of particular importance and has implications for whether effective vaccination can be achieved. Progress towards resolving these issues will be reported in the poster.

MI35 Multivalent salmonella vaccines: an *aroA* *msbB* *S. typhimurium* elicits a response to recombinant *P. yoelii* circumsporozoite protein

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Salmonella infections grow rapidly in mice, resulting in death once bacterial numbers reach a sufficient number. This toxicity is considered to be lipopolysaccharide related. A recently constructed *Salmonella* mutant with a deletion-insertion in its lipid A, *msbB* (*waaN*) gene was shown to elicit less inflammatory cytokines *in vivo*. Consequently, the mutant was able to grow to unprecedentedly high numbers in the host without causing death. This mutation was transduced into an *aroA*⁻ *S. typhimurium* vaccine strain and the subsequent double mutant was assessed for its ability to protect against virulent *Salmonella* challenge. The *aroA*⁻ *msbB*⁻ mutant was also assessed for its ability to elicit immune responses against recombinant antigens.

The circumsporozoite (CS) protein of the parasite *Plasmodium*, has attracted much attention as a candidate vaccine component against malaria. Using pTECH2, CS protein has been expressed in *Salmonella* as a fusion to the highly immunogenic but non-toxic TetC fragment of tetanus toxin. To overcome low expression, the CS gene was demarcated into three domains each containing distinct, immunostimulatory epitopes. Mice were immunised intravenously with *S. typhimurium* vaccine strains expressing either full length CS protein or the mixture of regions to determine protective immunogenicity.

The protective *aroA*⁻ *msbB*⁻ mutant elicited higher anti-CS protein antibodies than its *aroA*⁻ counterpart after a single dose and is therefore an attractive candidate for the delivery of foreign antigens in humans using *Salmonella typhi*.

MI36 *Neisseria lactamica* as a vaccine against meningococcal disease

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be involved in the development of natural immunity against meningococcal disease. *N. lactamica* has many surface structures in common with *N. meningitidis* and we have shown that *N. lactamica* antigens provide protection in an animal infection model of meningococcal disease.

The outer membrane proteins (OMP) of *N. lactamica*, extracted from whole cells using 0.3% elugent, were separated by semi-native preparative electrophoresis. The proteins were pooled into low (<43kDa, LMW), medium (43-67kDa, MMW) and high (>67kDa, HMW) molecular weight groups and used to immunise mice. The mice were challenged with *N. meningitidis*, strain K454, at two challenge doses. The LMW group provided the best protection. These proteins were further separated by preparative electrophoresis into group 1 proteins (<25kDa, g1), group 2 proteins (25-35kDa, g2) and group 3 proteins (35-43kDa, g3). Protection experiment results suggested that g2 and g3 proteins provided the best protection and sera raised against these proteins had higher bactericidal titres against *N. meningitidis* than for sera raised against the g1 proteins. Thus *N. lactamica* may have the potential to provide protection against meningococcal disease of all serogroups.

MI37 Identification of quorum sensing in *Burkholderia pseudomallei*

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Burkholderia pseudomallei is the aetiological agent of melioidosis. Melioidosis can form chronic or septicemic infections in man, with a fatality rate of 95 % in untreated cases. The treatment of melioidosis is complicated by the wide range of antibiotic resistance genes possessed by the organism. Antibiotic treatment reduces the fatality rate to 35 %, but 23 % of survivors will relapse within 5 years due to chronic infections. Such chronic infections can persist for decades. Due to the high level of resistance in this pathogen, it is desirable to identify novel targets for antimicrobial therapy which may be used to treat infections, especially chronic infections and intracellularly located bacteria. Quorum sensing systems have been identified as one such potential target. Quorum sensing is the ability of bacteria to regulate the expression of its genes in response to changes in their cell density. Many pathogens have been shown to regulate expression of their virulence genes using quorum sensing systems, mediated by N-acyl homoserine lactones (AHLs). In this study, we have identified a quorum sensing system present in *B. pseudomallei* and have cloned the genes involved, *BpmR* and *BpmI*, encoding the regulatory protein and the AHL synthase respectively. The AHL molecules produced by *B. pseudomallei* and the cloned *BpmI* have been purified and characterized.

MI38 *Salmonella typhimurium* and *salmonella typhi* as delivery systems for *Yersinia pestis* F1 antigen

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Attenuated intracellular bacteria such as *Salmonella* can be used as carriers of heterologous antigens to the immune system. The plasmid pAH34L, encodes the capsular F1 antigen of *Yersinia pestis*, the causative agent of plague. This plasmid has previously been transformed into the attenuated *S. typhimurium* strain SL3261 (*aroA*) and has been used to orally vaccinate mice. Protection was demonstrated against subcutaneous challenge with *Y. pestis*. The *Salmonella typhi* strain BRD 1116, attenuated by gene deletions in *aroA*, *aroC*

We have assessed the *in vitro* stability of the plasmid pAH34L, and the *in vitro* expression of F1.

MI39 Complete genomic analysis of the large 135kb pathogenicity island of *Salmonella typhi* CT18 and studies of the *viaB* operon located within it
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Salmonella typhi CT18 is a recent multiple drug resistant (MDR) clinical isolate obtained from a patient in Vietnam. Such *S. typhi* MDR strains increasingly arise due to anti-microbial overuse and exposure. A better understanding of *S. typhi* pathogenicity would be advantageous in tackling this increasing problem and could eventually lead to the development of more effective treatments. The sequencing of the entire *Salmonella typhi* genome was thus undertaken with this clinical isolate from Vietnam.

One of the characteristic features of *Salmonella typhi* pathogenicity is the expression of the VI polysaccharide capsule. The operon coding for this capsule, *viaB*, is located on a large 135kb pathogenicity island. Very little is known about the other genes found on this pathogenicity island which may also contribute to the unique clinical symptoms and host range of this bacterium. Genomic analysis of this PAI was carried out in order to elucidate what other genes may be present which may have roles in not only pathogenicity but also host attachment and transmission.

Analysis of the PAI showed the presence of a unique phage186- retron phage R86 hybrid that also contained the *sopE* gene. This complete phage was found less than 2kb downstream of the 3prime end of the *viaB* operon. Other unique genes or clusters are also described including genes that are related to type 4 secretion.

MI40 Rapid determination of the antibiotic susceptibility profiles of bacteria!
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Antibiotic-resistant bacteria are an increasingly serious problem. The traditional method of testing for susceptibility can take over 48 hours. We demonstrate here that a combination of antibiotic and bacteriophage-mediated cell lysis monitored by bioluminescence can allow antibiotic susceptibility to be determined in one hour. Cell lysis releases adenylate kinase (AK), which converts added ADP to ATP. Levels of ATP are then monitored using firefly bioluminescence. *E. coli* cultures were incubated with antibiotics and bacteriophages. Lytic antibiotics (e.g., ampicillin) rapidly lysed susceptible bacteria, masking any phage-mediated lysis. In resistant cultures no such lysis occurred, enabling the phage to complete their lifecycle (approximately 30 min). Luminescent signals resulting from phage-mediated lysis were much higher than background levels but lower than with antibiotic-mediated lysis, demonstrating that the bacteria were growing and thus ampicillin-resistant. Non-lytic antibiotics (e.g., chloramphenicol) caused minimal cell lysis in susceptible cultures because the bacteria died, preventing the bacteriophages from completing their lifecycles as an actively growing host is required in order to replicate. Resistant cultures showed considerable cell lysis 1h after incubation because the bacteriophage had been able to replicate and lyse host cells. Luminescence correlated with cell lysis, thus indicating whether a culture was resistant or susceptible to an antibiotic.

MI41 Sample sequencing of a total genomic library from *Francisella tularensis* strain Schu 4 reveals

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Francisella tularensis is the causative agent of tularemia, a serious and sometimes fatal disease. There is a live attenuated vaccine strain (LVS); but the molecular basis of attenuation is unknown and it is unlicensed. Therefore a need exists for a defined, characterized vaccine. To help obtain this goal we initiated a genome sequencing project with the virulent Schu4 strain of *F. tularensis*, which has to date produced a total of 1.83 Mb of nucleotide sequence in 353 contigs. Among the wide variety of genes that have been shown to be targets for inactivation in other bacterial pathogens in order to construct defined attenuated mutants, those in the aromatic amino acid (aro) or purine (pur) biosynthesis pathways have most often been focused on. We have identified genes which could encode the enzymes necessary for functional aro and pur pathways in *F. tularensis* Schu4. This data will be used to develop defined rationally attenuated mutants of *F. tularensis*, which could be used as replacements for the existing LVS.

MI42 The failure of *Yersinia pestis* to produce an O-antigen is due to frame shift mutations in the O-antigen gene cluster

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The lipopolysaccharide (LPS) of *Yersinia pestis* strain CO92 was purified using the phenol, chloroform, petroleum ether method. LPS purified by this method was devoid of an O-antigen side chain when analysed using SDS-PAGE. LPS from seven other strains of *Y. pestis* were found to be similar to that of *Y. pestis* strain CO92 when analysed by SDS-PAGE. The nucleotide sequence of *Y. pestis* strain CO92 revealed the presence of a putative O-antigen gene cluster. However, five frame shift mutations within the cluster may explain the absence of an O-antigen.

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Conjugate vaccines against *Neisseria meningitidis* serogroup C and *Haemophilus influenzae* type b (Hib) disease are licensed for use in the U.K. However, most conjugate vaccines currently use diphtheria or tetanus toxoids as the protein carrier, which does not broaden protection against childhood diseases and children already have maternal antibody to these proteins. In this study, *Bordetella pertussis* fimbriae have been assessed as novel carrier proteins for the capsular polysaccharides of *N. meningitidis* serogroup C (CPs) and Hib (PRP). *B. pertussis* fimbriae are not a component of some acellular pertussis vaccines and recent clinical trials have shown them to be important protective antigens against whooping cough. Fimbriae (2+3) were purified from *B. pertussis* strain Wellcome 28, and then denatured in guanidine-HCl to produce subunits of a defined size. Denatured fimbriae were shown to be protective against colonisation with *B. pertussis* in the mouse intranasal challenge model. Denatured fimbriae were conjugated to the bacterial polysaccharides by carbodiimide coupling or reductive amination. Denatured fimbriae and conjugates were characterised by gel filtration, chemical assay and immunoblotting. Following gel filtration chromatography, a molecular weight shift in the conjugated denatured fimbriae was observed. The conjugates were found to elicit a protective memory immune response in animal models.

MI44 The lysis of *Mycobacterium smegmatis* by mycobacteriophage in a model environmental microcosm

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The prevalence of bovine tuberculosis caused by *Mycobacterium bovis* is increasing, particularly in the Southwest of England where it affects more than 1% of herds each year. *M. bovis* is also known to infect badger populations and there is evidence to support the view that badgers are a significant source of infection for cattle.

Biological control using mycobacteriophage offers a potential strategy to control *M. bovis* in the environment without disrupting the ecosystem. Bacteriophage are self limiting, occur naturally in the environment and have a specific host range reducing the effect on other unrelated micro-organisms.

To determine the feasibility of using mycobacteriophage in the environment we have developed and employed a turf model microcosm that resembles pasture. This comprises rooted grass that has been excavated from farmland. It is held in a constant environment chamber where it can be exposed to controlled treatment regimes and periodically sampled. In initial studies the faster growing *Mycobacterium smegmatis* has been used. This has enabled the environmental model to be established, and parameters such as bacterial and phage survival to be more rapidly examined. Selective recovery of *M. smegmatis* from the turf model has also been achieved. Early indications are that the survival of *M. smegmatis* in the presence of bacteriophage is reduced and this approach therefore offers great potential.

MI45 Identification of genes involved in two-component signal transduction in *Burkholderia pseudomallei*

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organism is a facultative intracellular pathogen capable of remaining dormant in host macrophages for many years and the latency period can be activated to develop into an acute, fulminating and fatal infection when an individual becomes immunocompromised. *B. pseudomallei* is resistant to a large number of antibiotics and future therapeutic intervention could involve the development of a vaccine.

In this context, the aim of the project is therefore to identify and characterise appropriate genes and gene products, which may be suitable for generating mutants that could be used as live vaccines. Using degenerate oligonucleotide primers and *B. pseudomallei* genomic DNA, we have identified by PCR a DNA fragment which encodes part of an ORF that shows 66 % amino acid similarity to *Salmonella typhimurium* PhoP, a response regulator gene involved in two-component signal transduction. It has been previously shown that *phoP* mutations in *Salmonella typhimurium* and *Salmonella typhi* are attenuated and may be potentially useful as live vaccines. This PCR fragment has been used to screen a *B. pseudomallei* genomic library in phage to identify other *B. pseudomallei* genes involved in two-component signal transduction processes.

MI46 Stable and effective non-aqueous liquid tetanus vaccine

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The major block to the prevention of tetanus in the developing countries has been the lack of a heat-stable vaccine. Current vaccines are liquid formulations containing alum, the only adjuvant currently licensed for use in man, which foiled previous attempts to produce a stable, powder vaccine. A novel sugar-glass technology has been used to produce tetanus vaccine in a powdered form which, when re-suspended in non-aqueous biocompatible liquids, remained stable for three months at 37°C.

Commercially available, alum-adsorbed tetanus vaccine was powder-dried and suspended in both aqueous and non-aqueous liquid vehicles. Vaccine samples were tested for toxoid antigenicity *in vitro* and for immunogenicity *in vivo*. The formulation process caused some loss of detectable toxoid but the vaccines were still able to generate a protective antibody response *in vivo*, over a three month period. The antibody response to the alum-adsorbed vaccine fell significantly between 8 and 12 weeks whereas the responses to the stabilised vaccines remained elevated suggesting that the powdered vaccines were more stable *in vivo*. Storage at 37°C for three months caused a 75% loss of toxoid in the commercial vaccine but no such loss was seen in the stabilised powder vaccines. The latter retained antigenicity and immunogenicity after three months storage suspended in non-aqueous liquids.

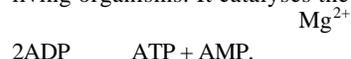
A stable liquid formulation for tetanus may help to bring about the prevention of neonatal tetanus in the third world and may lead to the development of other vaccines which will not require refrigeration to retain their potency.

MI47 Adenylate kinase as a cell-derived enzyme label in a magnetic bead immunoassay for the rapid detection of bacteria

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Adenylate kinase (AK) is an essential enzyme in virtually all living organisms. It catalyses the equilibrium reaction:



bacterial cells may be detected in a 5 minute assay, with increased sensitivity being obtained from longer assay times.

Here we describe the development of an immunoassay for the specific detection of Salmonella and *E.coli* O157 using AK bioluminescence. Target cells were captured using antibody-coated magnetic beads. These could be immobilised to allow removal of the sample matrix and for subsequent wash steps. A detergent-based extractant was used to release AK from captured cells so that purified ADP added at the same time could be converted to ATP in a linear amplification reaction. The resulting ATP was measured using the firefly luciferase reaction. Low concentrations of cells ($<10^3$ ml⁻¹ for Salmonella, $<10^2$ ml⁻¹ for *E.coli* O157) could be detected in about 10 minutes. Experiments to determine the optimum assay volume, incubation time and incubation temperature are described. The method is compared with a conventional colourimetric immunoassay.

MI48 Disseminated CTL responses to transgenes expressed from modified vaccinia virus ankara: influence of anti-vector antibody

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It is now widely accepted that a desirable feature of a vaccine against AIDS will be the ability to induce cytotoxic T lymphocytes (CTL). In this respect, immunisation with viral vectors offers great potential. MVA is a highly attenuated poxvirus with an impressive safety record. As part of a vaccine efficacy study, we sought to determine the ability of recombinant MVA expressing regulatory and structural genes of simian immunodeficiency virus (SIV) to induce CTL in macaques. A single vaccination induced responses to rev, tat and nef. Subsequent vaccination failed to reveal new specificities. Surprisingly, CTL directed against gag were not detected, even in two macaques with the MamuA*01 MHC type that restricts a gag epitope. Further investigation revealed that the MVA-SIVgag construct expressed only poorly. Upon further immunisation with an alternative construct, gag-specific CTL were detected despite pre-existing antibody to the vector. These responses were, however, short lived or weak compared to those induced in an animal with no pre-existing anti-vaccinia antibodies. Despite the systemic route of immunisation, high gag-specific CTL activity was detected in both systemic and local sites in the vaccinia-naïve animal but not in the vaccinia primed animals.

MI49 Detection of cold and hypochlorite stress proteins in *Salmonella typhimurium*

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Salmonellae are a major and persistent cause of bacterial food-borne illness in the UK and enter the food chain mainly through contaminated poultry and beef products. Commercial food preparation processes are designed to minimise the levels of such bacterial contamination of foodstuffs and also, their potential for replication. Consequently, microorganisms may either be killed or their growth inhibited by exposure to high levels of disinfection procedures used during industrial scale food processing or storage under conditions of refrigeration. Organisms such as *Salmonella typhimurium* respond to harmful environments by activating protective

specific proteins, which are induced. Such an approach is useful for furthering our understanding of the biological basis for microbial adaptation and survival. However, it may also be of practical value and provide an approach for validating the effectiveness on *S. typhimurium* of food-processing treatments. This study addresses the validity of such a strategy by focusing on the effects of exposure to refrigeration temperatures (approx. 4°C) or the bactericidal disinfectant, hypochlorous acid (HOCl). In order to exploit this strategy, 2-D gel electrophoresis coupled with nano-electrospray ionisation, tandem mass spectrometry and database searches were used for the rapid identification of proteins. This has allowed for the successful monitoring of the effects of these stresses in relation to growth. The study has shown that a selection of proteins respond to exposure to a specific stress while others remain relatively constant, under the conditions examined. The data presented in the poster reports the analysis of protein changes in response to cold and HOCl exposure. The biological nature of the proteins involved and practical applications of this approach will be also discussed.

MI50 Structure based vaccine design

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In a pathogen where virulence determinants are unclear it is common in the development of a vaccine to mutate genes encoding enzymes in a biosynthetic pathway which produce metabolites essential for pathogen survival. One such pathway which has attracted considerable interest is the shikimate pathway consisting of enzymes encoded by the *aro* genes. Early studies demonstrated that mutants of *Salmonella typhimurium aroA* generated by Tn10 transposon mutagenesis were highly attenuated in mice and were excellent single dose oral vaccines against challenge with the virulent parent strain. The *aro* genes are specific to bacteria, microbial eukaryotes, fungi, plants and parasites and are not metabolized by mammals, as such *aro* mutants are presumably attenuated because they are unable to obtain essential aromatic amino acids *in vivo*. Attenuation of virulence by mutation of *aro* genes has been successful in most instances. In this study we describe the cloning of *aro* genes from the bacterial pathogen *Francisella tularensis* (the causative agent of tularemia, a potentially fatal illness). A live vaccine strain exists in which the mechanism of attenuation of virulence is not understood, there is accordingly a requirement for a vaccine in which the nature of attenuation is rationally designed. Our group recently solved the crystal structure of *Aspergillus nidulans aroB* encoded dehydroquinate synthase (DHQS), the structure reveals which amino acids in the active site are involved in catalysis. We note that these active site residues are conserved in DHQS across species including *F. tularensis*. By deleting a 150 base pair stretch from *F. tularensis aroB* we have eliminated 5 such residues. In these studies we discuss the construction of a rationally designed *aroB* mutant as a candidate vaccine for tularemia.

MI51 Targetting mycobacterial promoters expressed *in vivo* by FACS analysis

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Mycobacterium tuberculosis, with 3 million deaths per annum, is responsible for more deaths than any other single pathogen. The slow growth of the organism and ability to

strains. The variable efficacy of the BCG vaccine coupled with the inadequacies of current antibiotic treatment make the development of improved vaccines and identification of new drug targets essential to effectively prevent and treat tuberculosis. Application of molecular microbiology to the pathogenesis of *M. tuberculosis* will facilitate identification of these novel therapeutic targets. We have employed green fluorescent protein (GFP) as a marker to identify and isolate promoters that are induced during mycobacterial infection and growth in macrophages. GFP is a silent marker of gene expression that allows dissection of the processes that occur during infection and has successfully been applied to other bacterial pathogens. Use of an ACDP 3 contained FACS sorter allows rapid analysis (and recovery) of large numbers of individual clones for GFP expression thereby facilitating whole-genome coverage. We are using a random promoter library and targeted individual promoters to reveal those which are induced *in vivo*.

MI52 Expression of truncated forms of recombinant PA and an evaluation of protective efficacy against *B. anthracis*

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Bacillus anthracis, the etiological agent of anthrax, secretes a tripartite toxin comprising protective antigen (PA), lethal factor and oedema factor. PA is a necessary immunogen in the current UK vaccine, which consists of an alum-precipitated cell-free filtrate of Sterne strain cultures grown to maximise the PA content. We have previously described the production and protective efficacy of a recombinant form of PA and solved the crystal structure. Both the native and recombinant crystal forms are organised into four distinct domains which are thought to be implicated in membrane insertion and receptor binding.

The DNA encoding the PA domains 1-258, 168-487, 168-595, 1-595, 1-487 and 1-735 from *B. anthracis* Sterne were PCR amplified and cloned into the *Xho*I and *Bam*H I sites of the expression vector pGEX-6-P3. *E. coli* expressing the GST fusion proteins were urea extracted (with the exception of domain 1-258) and purified batch-wise using glutathione Sepharose or Q Sepharose. Proteins were analysed by SDS-PAGE and Western blotting; and the tertiary structure confirmed using circular dichroism.

The purified GST fusion proteins will then be used to determine their protective efficacy against a subcutaneous challenge with *B. anthracis*, in the murine model and the immune response analysed.

MI53 The evaluation of DNA vaccines against aerosol challenge with *M. Tuberculosis* in guinea pigs

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There is a recognised need to develop improved vaccines to provide protection against tuberculosis in man and cattle. Given the cross-protection of BCG against human tuberculosis there is a rationale for evaluating the protection against *M. tuberculosis* and *M. bovis* afforded by vaccines derived from either species. In collaboration with the TB Research Group at the Veterinary Laboratories Agency, the efficacy of DNA coding for T-cell stimulatory antigens of *M.*

Protection against aerosol challenge with *M. tuberculosis* H37Rv was defined by assessing haematogenous spread to spleens and histopathology analysis of lung tissue, 10 weeks post-challenge. Compared with BCG or sham-immunised controls neither construct prevented colonisation of spleens. There were, however, differences between the vaccines in terms of number and type of lung lesion and the degree of caseation, fibrosis and calcification in lung sections. Animals vaccinated with pVR1020-85 showed a pattern of pathology similar to (but not as protective as) that caused by BCG immunisation. Animals vaccinated with pCMV83 had pathology scores similar to the sham-immunised controls. Thus, there was no cross-protection by DNA coding for a *M. bovis* antigen against *M. tuberculosis* infection.

MI54 Production of particulate-form recombinant subunit vaccine candidates against Japanese encephalitis virus (JEV) in insect cells

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Viral subunit vaccines with particulate structures have shown improved immunogenicity and protection efficacy when compared with monomeric antigens. Previous studies have demonstrated that extracellular subviral particles containing JEV prM, M and E proteins can be produced by Hela cells infected with a recombinant vaccinia virus and mice immunised with this particle are protected from lethal JEV infection. In the present work we have constructed a recombinant baculovirus encoding JEV prM and E genes and found that 1) insect cells (sf9) infected with this recombinant baculovirus can be labelled with monoclonal antibodies directed against JEV E protein by indirect immunofluorescence assay; 2) the recombinant baculovirus can induce cell fusion on sf9 cells; and 3) unique empty budding particles surrounding the cells infected with this recombinant baculovirus are revealed by electron microscopy. Purification and further characterisation of this particle are being carried out, which may represent an alternative approach to produce particulate subunit vaccine against JEV infection. In addition, we are expressing JEV prM and E genes in a nonlytic insect expression system (*Drosophila* Expression System) to overcome the adverse effect of cell lysis on expressed proteins. The stable expression cell lines have been established, and the formation of JEV subviral particles will be confirmed by electronic microscopy.

MI55 Investigation of coxsackievirus-like particles as vaccines in prevention of coxsackievirus-induced heart muscle disease

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Coxsackievirus B3 (CVB3) is a member of enterovirus in the picornavirus family. This virus, along with other enteroviruses, is involved in at least 50% of acute myocarditis case and in some cases of dilated cardiomyopathy (DCM), a leading cause of heart failure requiring heart transplantation. Development of a vaccine against CVB3 infection would have important economic consequences since. Potentially virus-like particles are far better immunogens than any other subunit vaccines as they are structurally similar to native virus particles. The formation of virus-like particles by other picornavirus has

like particles could be used to prevent myocarditis. The recombinant baculoviruses carrying the entire coding region of CVB3 were constructed and confirmed by PCR amplification, immunofluorescence and immunoblotting. Electron microscopy demonstrated CVB3 virus-like particles in a large cytoplasmic vacuole in insect cells (Sf9) infected with the recombinant baculoviruses. The virus-like particles were also observed in the culture medium. To confirm the formation of CVB3 virus-like particles, CsCl and sucrose gradient centrifugation and immuno-electron microscopic analysis are being carried out. We are assessing immunogenicity and protective effect of these virus-like particles on a murine model of CVB3-induced myocarditis.

M156 Probing the roles of key amino acid residues within the phospholipid-binding domain of *Clostridium perfringens* -toxin

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The main virulence factor produced by *Clostridium perfringens* in cases of gas gangrene is a phospholipase C. This phospholipase C (-toxin) also possesses haemolytic and lethal properties. The crystal structure of -toxin reveals that it is composed of 2 domains (N- and C-domains). The N-domain has phospholipase C activity whilst the C-domain if the calcium-dependent putative phospholipid binding domain. The *Clostridium bifermentans* phospholipase C (Cbp) shows amino acid sequence identity but is non-toxic. We have identified key amino acid residues which are thought to be involved in phospholipid binding by -toxin. These residues are not conserved in *C. bifermentans* Cbp and may influence the properties of this enzyme. Site-directed mutagenesis was used to replace these residues in -toxin with their counterparts in *C. bifermentans* Cbp. The effect on phospholipase C and haemolytic activity was determined.

M157 Detection of pathogenicity and heat stable toxin production in strains of *Yersinia enterocolitica* using Polymerase Chain Reaction (PCR)

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93 *Y. enterocolitica* strains were examined for pathogenicity characteristics using both conventional methodology and a PCR technique targeting the pathogenic all *ail* and *yst* genes. The isolates examined consisted of both human isolates and bovine milk strains.

The objectives of the study were:

- To determine the pathogenic status of the isolates
- To determine whether the bovine isolates which had previously been diagnosed as toxin producers by the mouse test had the *yst* gene
- To compare tests for phenotypic markets *viz.* Ca dependency, autoagglutination, Crystal Violet Staining and pyrazinamidase activity with genetic pathogenic markers *viz.* the *ail* and *yst* genes.

Results and discussion

Serovar 0:5 *Y. enterocolitica* strains isolated from human patients suffering from diarrhoea did not show any phenotypic signs of pathogenicity and not possess either the *ail* or *yst* genes. These observations raise questions about the role of the 0:5 serovars as agents of disease. It may be that although *Y. enterocolitica* was isolated from

The 15 human isolates, serotypes 0:3 and 0:9, which produced virulence characteristics by conventional methodology were shown to have the *ail* and *yst* genes by PCR. 0:3 and 0:9 serovars are widely reported as the main pathogenic serovars in Europe.

A plasmidless variant of the 0:3 serotype which showed the presence of the *ail* and *yst* genes, still lacked pyrazinamidase activity but no longer showed conventional pathogenicity characteristics. The *ail* and *yst* genes are located on the main chromosome and are therefore not lost, while the plasmid encoded conventional virulence markers are lost with the virulence plasmid. The *pyr* gene which is located on the main chromosome is reported to correlate with a strains inability to harbour the virulence plasmid. Thus pyrazinamidase positive strains will not have the virulence plasmid and its associated virulence characteristics.

The pyrazinamidase test should be included when testing for plasmid markers.

The 26 bovine isolates which had already been tested for enterotoxin production using the mouse assay (Walker & Gilmour, 1990) lacked the *yst* gene encoding for toxin production. This suggests that the mouse assay was not detecting the presence of heat stable enterotoxin but some other characteristic. In addition, these bovine isolates did not show any of the other conventional, phenotypic markers of pathogenicity and were pyrazinamidase positive which correlates with the cells inability to harbour the virulence plasmid.

Acknowledgments: the authors are indebted to Dr Ken Liddel of Law Hospital Lanark for his co-operation and supply of clinical isolates and to Professor Arthur Gilmour of Queens University Belfast for the bovine milk isolates. This research was supported by Scottish Office funding under ROAME 644055 Commission No SAC/024/93.

M158 Expression and mutagenesis of an aminoglycoside nucleotidyltransferase reveals an important aspartate residue

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The aminoglycoside antibiotics are a large class of important antibiotics which have uses in the treatment of many different infections. Bacterial resistance to the aminoglycosides can be through the action of a phosphotransferase, an acetyltransferase or an adenylyltransferase. The antibiotic resistance gene, aminoglycoside(3i)nucleotidyltransferase (ant(3i)-Ia), which confers resistance to spectinomycin and streptomycin is present in many resistance plasmids and transposons. We have expressed this enzyme and determined some molecular properties of the enzyme. Mutagenesis has revealed the importance of an aspartate residue.

The ant(3i)-Ia originally from the plasmid R538-1 was isolated on a 1.95Kb HindIII restriction fragment from plasmid pIJ4642. To enhance expression of ant(3i)-Ia, the gene was further cloned into a variety of expression vectors namely pUC19 (lac) pTTQ19 (tac), pMTL2023 (trp) and pMTL1005 (mdh).

The enzyme was subsequently purified to homogeneity by a combination of ammonium sulphate precipitation, ion exchange chromatography, hydrophobic interaction chromatography and gel filtration steps. Kinetic and molecular characteristics of the enzyme have been determined and the Km and Vmax have been determined for ATP, streptomycin and spectinomycin.

Comparative amino acid sequence homology studies have been performed with other members of ANT(3i)-I enzymes and nucleotidyltransferases. This has revealed extensive homology throughout, although certain regions of the gene appear to be more highly conserved than others. In order to test the biological importance of these regions, site directed mutagenesis has been carried out to alter some highly conserved aspartic acid residues, the ability of mutant enzymes to confer resistance to antibiotic substrates was evaluated.

M159 Human vaccine against anthrax

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Vaccination is acknowledged as the most cost effect form of

concerning residual pathogenicity, live spore vaccines have not been adopted for human use in the UK and US.

The current UK human anthrax vaccine has been in use since 1963 and during that time over 50,000 doses have been given. The vaccine consists of a protein precipitate from the supernatant of cultures of the Sterne strain. The major immunogen is protective antigen (PA) the non-toxic, cell-binding component of the anthrax toxin complexes.

In an attempt to better understand the basis of the human immune response to the vaccine, the PA-specific antibody response of individuals given their annual booster dose was determined. The concentration of anti-PA IgG antibodies, determined by ELISA, was found to peak at 3380 μ g/ml (\pm 914; n=7), 14 days post vaccination and returned to preboost levels approximately 18 weeks later.

To further characterise the specificity of the human antibody response to PA, the ability of human polyclonal sera to inhibit the binding of PA specific monoclonal antibodies was determined.

WEDNESDAY 12 APRIL 2000

0920 Nucleosome positioning dictates amino terminal tail and histone acetylation-dependent activation of the PHO5 promoter

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Histone acetylation plays a key role in chromatin dynamics, and it has been widely assumed that acetylation of key lysine residues within the histone tails will accompany the chromatin remodeling required for transcription activation at most if not all promoters. Induction of the PHO5 promoter is characterised by a chromatin transition resulting in the remodeling of 4 precisely positioned nucleosomes. Nucleosome positioning, chromatin remodeling and transcription activation at the chromosomal PHO5 promoter are all entirely independent of acetylation of the histone H4 and H3 tails. The integrity of the H3 or H2B tails are also dispensable for regulation of PHO5. In contrast, under conditions that result in imprecise nucleosome positioning over the PHO5 promoter, dependence on the integrity of the amino terminal histone tails is observed. Transcription from a plasmid based PHO5-lacZ reporter, which exhibits subtle perturbations to the chromatin structure, is absolutely dependent on the histone H4 tail, while the amino terminal tail of H2B is implicated in the inefficient derepression of PHO5 in the *pho80/gen5* background. Thus, chromosomal PHO5 provides a clear example of a gene for which the underlying chromatin organisation dictates its dependence on the amino terminal tails of specific histone proteins.

0955 Activation of transcription by small molecules
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The yeast *Saccharomyces cerevisiae* responds to being presented with galactose as the sole source of carbon by activating the genes encoding the enzymes of the Leloir pathway. This process depends on a transcriptional activator, Gal4p. In the absence of galactose, a repressor, Gal80p, inhibits the activity of Gal4p. The switch from repressed to active transcription involves another protein, Gal3p. Galactose, and ATP, allows an interaction between Gal3p and Gal80p to occur. This results in the formation of a transcriptionally active Gal4p-Gal80p-Gal3p complex. The precise mechanism of Gal3p function is unclear. Gal3p is highly homologous (70% identical and 90% homologous at the amino acid level) to Gal1p, the galactokinase, although it possesses no galactokinase activity of its own. We have studied the switch between repressed and activated transcription in the *GAL* system using purified proteins to elucidate the mechanism of galactose induction. We find that the insertion just two amino acids from an appropriate region of Gal1p will convert Gal3p into a galactokinase. These, and other biochemical data, will be discussed in terms of the mechanism of *GAL* induction.

1100 Regulatory circuits controlling nitrogen metabolism in yeast

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Preferential utilization of good nitrogen sources (glutamine) over poor ones (proline) is accomplished by the GATA-family DNA binding proteins in a process designated as nitrogen catabolite repression (NCR). NCR-sensitive transcription is regulated in two different, but inter-related ways. The first is competition between the transcriptional repressors (Dal80p & Deh1/Gzf3p) and activators (Gln3p & Gat1p/Nil1p) for binding to GATAA-containing UAS_{NTR} elements upstream of NCR-sensitive genes; this mode is highly sensitive to the relative amounts of the respective GATA-transcription factors and their regulation of one another's production. The second, and more dominant form of regulation is via Ure2p-mediated inhibition of the ability of Gln3p and Gat1p to activate transcription in the presence of excess nitrogen. Inhibition occurs through Ure2p-Gln3p, Ure2p-Gat1p complex formation. With glutamine as nitrogen source, the GATA sequences of some NCR sensitive genes can be shown to be unoccupied by GATA-factors. Under these circumstances EGFP-Gln3p and EGFP-Gat1p are restricted to the cytoplasm. When NCR-sensitive genes are being highly expressed, the GATA activation factors are predominantly nuclear. These data suggest that under conditions of NCR, Ure2p complexes with Gln3p and Gat1p thereby preventing their entry into the nucleus and hence access to their target binding sites. Supported by NIH grant GM-35642.

1135 Regulation of stress responses in yeast

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Transcriptional responses of *Saccharomyces cerevisiae* to stress are mediated by several transcription factors, among them heat shock factor, Msn2 and Msn4, and Yap1. Msn2 and Msn4 are two redundant factors involved in the yeast general stress response. They are activated by a broad variety of stress factors, at least many of them acting primarily at the level of the plasma membrane. This will then cause, in a yet unknown way, nuclear import of Msn2/4, simultaneously downregulating nuclear export and, independently, specific STRE binding, thereby inducing transcription. Expression of more than 50 Msn2/4-regulated genes will then contribute to the general stress response. Protein kinase A (PKA) powerfully downregulates Msn2/4. It acts by specifically inhibiting nuclear import and STRE binding, and by inducing nuclear export. According to our present model, all these effects are caused by direct phosphorylation at specific sites. By counteracting Msn2/4 action, PKA also represses expression of some negative regulators of cell growth like the Yak1 protein kinase, thereby providing a direct link between negative stress control and positive control of cell growth.

1400 Transcriptional regulation in response to pH in

Aspergillus

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Extremes of pH are an occupational hazard for many

syntheses of molecules functioning beyond the cell boundary such as permeases, secreted enzymes and exported metabolites to the pH of the growth environment. The transcriptional regulator PacC, which contains three C₂H₂ zinc fingers, mediates such pH regulation in the fungus *Aspergillus nidulans* in response to a signal provided by the products of the six *pal* genes at alkaline ambient pH. In the presence of the alkaline pH transduction signal, the full length 674 residue form of PacC is proteolytically cleaved to yield the functional form, containing the ~249 N-terminal residues, which activates expression of genes expressed at alkaline ambient pH and prevents expression of genes expressed at acidic pH. Loss-of-function mutations in the six *pal* genes of the pH signal transduction pathway mimic the effects of growth under acidic conditions. The products of two of the six *pal* genes are predicted to contain transmembrane domains and a third probably encodes a cysteine protease of the calpain family, albeit not the protease responsible for the final processing of PacC to the functional form.

1435 Developmental gene transcription in *Candida albicans*

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C. albicans responds to certain environmental signals by changing its growth between a yeast and a hyphal form. Other differentiation events lead to alterations in colony morphology, such as the *white-opaque* phenotype in strain WO-1 (phenotypic switching) and to the formation of chlamydospores. Our results indicate that the bHLH transcription factor Efg1p regulates all of these morphogenetic processes.

The lack of Efg1p renders cells unable to form hyphae in most media containing inducers including serum and N-acetylglucosamine and also prevents the formation of chlamydospores. Low *EFG1* expression is correlated with an elongated, *opaque*-like phenotype, in which an *opaque*-specific transcript, *OP4*, is induced, while forced *EFG1* expression leads to the *white* phenotype, which grows as a typical yeast. Thus, Efg1p appears to be also a key factor in phenotypic switching.

A direct target for Efg1p has not yet been identified, although Efg1p is required for the expression of several hypha-specific genes. We have discovered that *EFG1* is autoregulated, suggesting that its promoter region contains sequences mediating Efg1p-dependent regulation. Transcriptional analyses revealed that *EFG1* is transcribed into a major transcript containing an extensive 1.16 kb untranslated region, and a minor transcript initiating just upstream of the ATG. Deletion analyses revealed that both the major and minor transcript are independently autoregulated, being repressed at high Efg1p levels. Surprisingly, a single E-box-like sequence within the minor *EFG1* promoter was not necessary for autoregulation, suggesting that as in the case of *myc* autoregulation, bHLH-E-box interaction is not the basis of autoregulation.

1600 Transcriptional control during mating and development in *Ustilago maydis*

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In the phytopathogenic fungus *Ustilago maydis* the multiallelic *b* mating-type encodes a pair of unrelated homeodomain proteins termed bE and bW. When originating from different alleles, the proteins form a heterodimer that is presumed to regulate pathogenicity genes, either directly by binding *tocis* regulatory sequences, or indirectly via a *b*-dependent signal cascade.

Using a fusion protein consisting of bE and bW we were able to identify the first direct target gene for the bE/bW heterodimer. The heterodimer binds specifically to a sequence

In a screen for components of the *b*-dependent signal cascade we have isolated two different genes, coding for a histone deacetylase (Hda1) and protein with similarities to the human retinoblastoma binding protein 2 (Rum1). Both genes are essential for the establishment of a repressed state of several *b* regulated genes in the absence of the *b* heterodimer. We propose that both Hda1 and Rum1 form a complex with other proteins with at least one of them allowing sequence specific DNA binding. In this model, the regulation of gene activity is achieved by modulation of the chromatin structure mediated by the action of the histone deacetylase Hda1.

1635 Internuclear gene silencing in *Phytophthora infestans*

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Transformation of the diploid oomycete *Phytophthora infestans* with antisense, sense and promoter-less constructs of the coding sequence of the elicitor gene *infl* resulted in transcriptional silencing of both the transgenes and the endogenous gene. Mycelial cells of *P. infestans* may contain multiple nuclei, resulting in heterokaryotic strains. Here, we demonstrate that: (i) transcriptional gene silencing is dominant in multinucleated cells, (ii) the silenced state can be transmitted from nucleus to nucleus in heterokaryotic strains, and (iii) gene silencing is maintained in a non-transformed nucleus after nuclear segregation. Our results indicate the involvement of internuclear transfer of signals from silenced nuclei to wild type nuclei, leading to stable gene silencing in the wild type nuclei. Once gene silencing has been induced in a wild type nucleus, it is maintained in progeny containing only the wild type nuclei. Our findings support a model reminiscent of paramutation and involving a *trans*-acting factor that is capable of transferring a silencing signal between nuclei. Here we present and discuss new experimental data of the mechanism of internuclear gene silencing in *P. infestans*.

POSTERS:

PBMG01 Regulation of transfer genes in the broad host-range antibiotic resistance plasmid RK2

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The broad host-range plasmid RK2 carries multiple antibiotic resistance genes. Conjugative transfer of plasmids such as RK2 is an important mechanism allowing the spread of antibiotic resistance genes from one bacterial strain to another.

Expression of genes required for conjugative transfer of RK2, found in the Tra1 and Tra2 regions, is controlled by global repressor protein TrbA. Defining the binding site for this protein will allow us to understand better the circuits which control transfer of this plasmid. A consensus sequence for the TrbA operator binding site has been proposed (Pansegrau & Lanka 1996 Prog. Nucleic Acid Res. Mol. Biol. 54: 197-251) based on sequence similarities in regions which are known to be subject to TrbA repression (Zatyka *et al.* 1994 Microbiology. 140: 2981-90). To test this hypothesis we have focused on the Tra2 promoter *trbBp*, where we have introduced point mutations within the proposed operator sequence and flanking nucleotides and measured their effect on promoter sensitivity to TrbA regulation. Results so far

sequence; results from experiments designed to assess the importance of adjacent bases will be reported.

At *trbBp*, TrbA interacts co-operatively with a second repressor, KorB, which binds at a site >150 bp away. KorB also binds at sites in the Tra1 region at some distance from TrbA-regulated promoters. We propose that these sites may also be involved in co-operative regulation of transfer genes by KorB and TrbA. This hypothesis is being tested by *in-vivo* assays of sensitivity to the two regulatory proteins for each of the three promoters in this region, in combination with the various promoter-distal KorB operators. Current results will be presented.

PBMG02 Proteolysis and the control of dinuclear iron active-site monooxygenases

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Soluble methane monooxygenase (sMMO) from *Methylococcus capsulatus* (Bath) and alkene monooxygenase (AMO) from *Rhodococcus rhodochrous* (formerly *Nocardia corallina*) B-276 are homologous multicomponent enzymes that catalyse NAD(P)H-dependent oxygenation reactions. sMMO and AMO each have three components: (1) a dinuclear iron active-site oxygenase that is the site of substrate oxygenation; (2) an NAD(P)H-dependent reductase with a flavin nucleotide prosthetic group and an Fe₂S₂ cluster; (3) a small polypeptide, known as protein B or the coupling protein, without prosthetic groups that is also required for full activity. In *M. capsulatus* (Bath) growing on methane under conditions of copper limitation, sMMO catalyses the conversion of methane to methanol, which is the first step in methane metabolism. Likewise, when *R. rhodochrous* B-276 grows using propene as sole carbon and energy source, AMO catalyses the first step in propene metabolism, which is the conversion of propene to R-epoxypropane. The protein B/coupling protein components of AMO and sMMO are both highly sensitive to inactivation by proteolysis and so proteolysis may be important in regulating the principal metabolic steps that both enzymes catalyse.

The protein B component of sMMO appears to be necessary to allow flow of reducing equivalents from the reductase to the hydroxylase. It undergoes apparently spontaneous proteolytic cleavage to remove 12 amino acids from the N-terminus, yielding the truncated protein B'. B' still binds to the hydroxylase component but does not activate substrate oxygenation. By using site-directed mutagenesis and other genetic methods, we have identified structural features that are required for protein B activity and which make it susceptible to specific proteolytic inactivation. Protein B' has been observed *in vivo*, suggesting that it is physiologically significant. Its formation may prevent nonproductive consumption of NADH or formation of harmful reactive oxygen species when substrate is limiting.

We are also investigating the homologous coupling protein component of AMO in order to understand the structural basis and physiological significance of its proteolytic inactivation.

PBMG03 *In vitro* degradation of glycoprotein glycans by pneumococci and subsequent analysis of protein expression

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Streptococcus pneumoniae has been shown to degrade and utilise the glycans present in two glycoproteins, human

medium where growth was dependent upon the addition of a source of carbohydrate e.g. glucose, AGP or fetuin. The changes to the glycans occurring during growth were investigated by monosaccharide analysis of culture supernatant by acid hydrolysis and high pH anion exchange chromatography (HPAEC) analysis. To investigate the changes in gene expression associated with growth on glycoprotein substrates, cells were harvested from mid-exponential phase cultures in defined media containing either glucose or fetuin and analysed by 2-dimensional electrophoresis (2-DE). Cellular proteins were separated by 2-DE, stained and the observed patterns of separation compared. Most of the approximately 400 separated polypeptides observed matched between the two cultures. However, several differentially expressed polypeptides were detected on 2-DE gels derived from cells grown on fetuin. The use of matrix assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-ToF-MS) to generate peptide mass-fingerprints and map these peptides to the *S. pneumoniae* genome is discussed.

PBMG04 Prodigiosin, carbapenem antibiotic and exoenzymes: quorum sensing in *Serratia* sp

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Serratia sp. strain ATCC 39006 ('39006') produces the bright red pigment, prodigiosin (Pig), and a carbapenem antibiotic, 1-carbapen-2-em-3-carboxylic acid (Car). Previously, it was shown that synthesis of these secondary metabolites is coordinately regulated by the global regulator protein Rap (regulator of antibiotic and pigment). This present investigation has shown that 39006 also produces two *N*-acyl homoserine lactones (putatively identified as *N*-butanoyl-L-homoserine lactone and *N*-hexanoyl-L-homoserine lactone), diffusible signalling molecules associated with the phenomenon of quorum sensing (cell-to-cell communication in bacteria). *smal* mutants, deficient in the production of these signalling molecules, were deficient in the production of Pig, Car, 'white-opaque phenotype' and the synthesis of the exoenzymes pectate lyase and cellulase. The regulation of Pig and Car with respect to Rap and quorum sensing has been investigated in this study using *lacZ* transcriptional fusions to *pigA*, *pigH* (Pig biosynthetic cluster) and *carA* (Car biosynthetic cluster).

PBMG05 Expression of multiple enzymatically active forms of sialidase by *Streptococcus pneumoniae*

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Sialidase activity has been proposed as a major virulence determinant for *Streptococcus pneumoniae* and the interaction of this organism with the oligosaccharide moieties of glycoproteins is well established. We have developed an *in vitro* growth model in which bacterial proliferation is dependent upon the degradation and utilisation of N-linked, sialylated glycopeptides. *S. pneumoniae* sialidase activity was induced by growth either on this substrate or on free N-acetylated amino sugars, with negligible activity found in glucose-grown cultures. Enzyme preparations derived from glycopeptide-supplemented cultures were analysed by non-denaturing polyacrylamide gel electrophoresis and we have demonstrated the presence of four major bands corresponding to distinct sialidase activities which were active against a substrate containing 2,3-linked N-acetylneuraminic acid. This pattern of sialidase expression differed markedly to that obtained when *S. pneumoniae* was cultured in nutrient-rich, commercially supplied media. Examination of the genomic sequence of *S.*

vitro. Data on the further biochemical characterisation of these enzymatically active forms of the *S. pneumoniae* sialidases will be presented.

PBMG06 Production of proteolytic enzymes during development of the actinomycete, *Micromonospora echinospora*

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Members of the genus *Micromonospora* exhibit complex developmental cycles, differentiating both morphologically and physiologically. The physiology of the genus is poorly understood when compared with other actinomycete genera such as *Streptomyces*. It is believed that a greater understanding of *Micromonospora* physiology will lead to increased industrial potential for the genus. This particular study has focused on the production of proteolytic enzymes in the gentamicin producer *Micromonospora echinospora*. The production of extracellular proteolytic enzymes was studied in a complex liquid medium containing yeast extract and peptone. A rapid increase in extracellular proteolytic activity was observed prior to sporulation and increased throughout the stationary phase. Preliminary inhibitor and electrophoresis studies indicate the presence of multiple proteolytic enzymes, with their pH optima lying between pH 6.5 and 8.5.

This work may provide further evidence for the role of proteolysis in the developmental systems of actinomycetes.

PBMG07 Cloning of a gene encoding a C₂H₂ zinc finger protein that complements a salt sensitivity phenotype in *Aspergillus nidulans*

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We report the cloning and sequencing of a gene encoding a putative C₂H₂ Zinc finger protein from *Aspergillus nidulans*. The DNA sequence was cloned by complementation of the salt sensitive phenotype of the *A. nidulans* *sltA1* mutation. A complementing 3.9 kb *Pst* I fragment contained one large open reading frame of 1554 bp. The predicted protein (STZA) comprises 517 amino acids and possesses three Zinc fingers along with a highly acidic domain. The Zinc fingers show highly conserved motifs with a number of transcription factors including the At hook protein from *Drosophila melanogaster*, CREA from *A. nidulans* and the human Wilm's tumour susceptibility protein WT-1. It is proposed that STZA has a role in the response of *A. nidulans* to a variety of environmental stresses.

PBMG08 Characterisation of a STRE binding protein gene from *Candida albicans*

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Yeast-to-hypha morphogenesis in *C. albicans* can be triggered by a range of conditions *in vitro*, many of which

Leng, *et al.* (2000) in preparation], revealed sequences that are identical to the **ST**ress **R**esponsive **E**lement (STRE) consensus sequence in *Saccharomyces cerevisiae* (C₄T). Taken together, these observations suggest that there might be a link between stress responses and morphogenesis in this pathogenic fungus. Gel mobility shift assays indicated that *C. albicans* protein(s) bind STRE in a sequence-specific manner. Hence, we screened our *C. albicans* lambda ZAPII cDNA expression library [Swoboda, *et al.* (1993) *Infection & Immunity* **61**, 4263] for clones expressing proteins that bind [³²P]-labelled STRE-containing oligonucleotides in a sequence-specific fashion. One such cDNA clone was used to isolate its cognate gene by colony hybridisation. Both the cDNA and gene clones were sequenced, revealing an ORF capable of encoding a protein of 309 amino acids containing a C₂H₂-zinc finger motif near its C-terminus. The zinc finger domain of this protein displays the highest sequence similarity to *S. cerevisiae* Nrg1p (67% identity), and hence the gene was named *CaNRG1*. In *S. cerevisiae*, *NRG1* mediates glucose repression of the *STAI* (glucoamylase) by recruiting the Ssn6p-Tup1p complex. Deletion of *CaNRG1* results in constitutive pseudohyphal phenotypes in *C. albicans*, under conditions that normally promote growth in the yeast form. This is consistent with the idea that Nrg1p represses filamentous growth, possibly by recruiting Tup1p [Braun and Johnson (1997) *Science* **277**, 105].

PBMG09 Endoglycosidase production by enterococci

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Enterococci are major nosocomial pathogens and are responsible for a wide variety of infections ranging from urinary tract infections to bacteremia and infective endocarditis.

Studies on *Enterococcus faecalis* have shown the production of an extracellular endo- N-acetylglucosaminidase activity which cleaves high-mannose type glycoproteins between the two N-acetylglucosamine residues of the pentasaccharide core. The released glycans were subsequently degraded by cell-associated mannosidase activities, liberating free mannose which supported bacterial growth. No free glycans smaller than Man₅-GlcNAc were detected in the culture supernatant during bacterial growth. We have investigated the distribution of this endoglycosidase activity within the genus enterococcus using ribonuclease B, a model glycoprotein which possesses one N-glycosylation site hosting a family of five high-mannose type glycoforms. Following bacterial incubation culture supernatants were prepared and the residual glycoprotein analysed directly by MALDI-ToF mass spectrometry. Liberated glycans, where present, were resolved by HPAEC to confirm the endoglycosidase cleavage specificity. We have found that over half the enterococcal species contained this endoglycosidase activity, the most notable exception was the lack of this activity in *Enterococcus faecium* isolates. The majority of these endoglycosidase-producing enterococci were able to utilise the released carbohydrate from ribonuclease B to support bacterial growth, suggesting a mechanism similar to that found in *Enterococcus faecalis*.

PBMG10 The role of peptide metabolism of *Streptococcus uberis* in growth in milk

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Infection with *Streptococcus uberis* accounts for around 33% of all cases of bovine mastitis in the UK. This bacterium is

bovine plasminogen to the caseinolytic enzyme plasmin, and can utilise peptides derived from bovine caseins by the action of plasmin. The link between the utilisation of these peptides and the ability to grow in milk and/or the bovine mammary gland has not yet been established.

A mutant strain of *S. uberis* 0140J, unable to utilise an essential amino acid from a beta-casein derived oligopeptide, was shown to contain a lesion within a gene which showed homology to an oligopeptide permease gene (OppF) of *Streptococcus pyogenes*. This strain was also unable to utilise Glutamic acid-containing di- and tripeptides, and differed in its ability to utilise larger peptides. Growth yield of the mutant strain in skimmed milk was 10-fold lower than that of the wild-type, which was restored in the presence of amino acids. Further investigation into this route of nutrient acquisition may identify key processes and bacterial molecules that enable *S. uberis* to grow *in vivo*.

PBMG11 Determination of extracellular and cell-associated factors affecting the secretion of *Bacillus anthracis* protective antigen from *Bacillus subtilis*
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The major protective immunogen in the current licensed UK human vaccine against *Bacillus anthracis* is based on protective antigen (PA), a component of the anthrax toxin. Although the Sterne strain from which the vaccine is produced is avirulent, it is still regarded and handled as an ACDP category 3 pathogen. Consequently a vector system has been developed to enable the expression of the PA gene (*pag*) in *B. subtilis*.

However, as with many other heterologous proteins, the rPA produced by *B. subtilis* is prone to rapid degradation by host cell proteases, especially during stationary phase. Consequently the rPA must be harvested towards the end of exponential growth, limiting product yield. We have devised a systematic approach towards increasing the current yield of rPA from *B. subtilis*. This is based on determining the influence of cell-associated determinants including protein folding factors (extracellular foldases and metal cations) and wall-associated proteases, as well as the effects of individual extracellular proteases.

PBMG12 Proteomic analysis of the acid response of *Streptococcus mutans*

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Streptococcus mutans is the principle causative agent of dental caries. Preliminary studies had demonstrated that, in addition to the ability to survive acidic conditions, *S. mutans* could grow at pH levels as low as 5.2. To identify proteins required for growth of *S. mutans* at low pH *S. mutans* strain UAB159, currently being sequenced at the university of Oklahoma, was grown in nutrient rich media adjusted to pH 7.0 and pH5.2. Whole cell proteins were extracted and have been compared. Preliminary mapping of cell extracts, from two dimensional (2D) gels using gel analysis software (Phoretix), has shown that several proteins are newly expressed during growth at low pH, indicating that metabolic pathways are switched on and acid shock proteins synthesised to enable the cells to tolerate the stressed acidic environment. It has been demonstrated that both commercially available and streptococcal proteins from 2D gels at low concentrations (1 picomole on the gel) can be extracted and digested for mass spectrometric analysis with subsequent identification of the proteins by peptide mass fingerprinting and comparison with genomic databases. With

and these strategies are currently being developed to identify those proteins up-regulated as a response to growth at low pH.

PBMG13 Regulation of the *HYR1* and *ALS7* genes during *Candida albicans* morphogenesis

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We have isolated and characterised two hypha-specific genes from *C. albicans*. Both *HYR1* and *ALS7* appear to encode non-essential GPI-anchored cell surface glycoproteins. The induction of their mRNAs correlates with hyphal development under at least three different inducing conditions: (a) temperature elevation and serum addition, (b) temperature and pH elevation, and (c) *N*-acetylglucosamine. Computer analysis of the 5' -regions of these genes reveals sequences that are identical to enhancer elements in other fungi. Interestingly, given the roles of nitrogen starvation stresses, and cAMP in the induction of hyphal growth, these sequences include Gcn4p Response Elements (GCRE), general Stress Response Elements (STRE), and cyclic AMP Response Elements (CREB). Each of these elements forms sequence-specific complexes with *C. albicans* cell free extracts *in vitro*, and these elements are present in the region of the *ALS7* promoter required for hypha-specific gene activation of *RLUC* gene fusions. We have analysed the induction of the *ALS7* and *HYR1* mRNAs in morphogenic signalling mutants. These confirmed the tight correlation between *HYR1* and *ALS7* expression and hyphal development. We show that the activation of these genes depends upon Efg1p, and that Efg1p binds directly to the E-box, copies of which lie within *HYR1* and *ALS7* promoters. However, an E-box is not sufficient to confer hypha-specific gene activation. Also, *HYR1* and *ALS7* are repressed by Tup1p. We present a model for hypha specific gene activation in which morphogenetic regulation by Efg1p and Tup1p is integrated with other environmental inputs, for example amino acid starvation and stresses.

PBMG14 *Bordetella pertussis* *lpxA* has both C10 and C14 acyl chain substrate specificity

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LpxA is a UDP-GlcNAc acyltransferase involved in lipid A formation in Gram negative bacteria. *LpxA* transfers an acyl chain from acyl carrier protein to UDP-GlcNAc. LipidA is derived from two acylated GlcNAc precursors and thus contains two *LpxA* transferred acyl chains. In a large majority of lipidAs, these two acyl chains are identical and this correlates with studies which demonstrate that the *LpxA* enzyme from several species have a single acyl chain substrate specificity. *B. pertussis* expresses an unusual lipidA in which one of the *LpxA* transferred acyl chains is C14 whereas the other is C10. This raises the possibility that *B. pertussis* either expresses two *lpxA* genes whose products have different substrate specificities or expresses a single gene whose product has dual substrate specificity. We have cloned and expressed *B. pertussis lpxA*. We demonstrate that the resulting enzyme has both C14 and C10 activity. We discuss these findings with respect to models of lipidA biosynthesis and with respect to *B. pertussis* biology.

PBMG15 REGULATION of calcium-dependent antibiotic (*cda*) production by *Streptomyces coelicolor* A3(2) and *Streptomyces lividans* 1326

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The calcium-dependent antibiotic (CDA) is produced by *Streptomyces coelicolor* A3(2). The *cda* biosynthetic gene cluster has been identified and occupies approximately 85 kb at the 10 o'clock region of the chromosome.

A DNA fragment encoding a positive regulator of CDA production, designated CdaR, has been cloned. *S.lividans* 1326 contains the *cda* gene cluster but doesn't normally produce CDA. S1 nuclease protection experiments have revealed that in *S.lividans* 1326 the level of *cdaR* transcription is significantly reduced when compared to that of *S.coelicolor*. A 0.8 kb in-frame deletion was made in *cdaR*. This deletion abolishes CDA production indicating that CdaR is essential for CDA biosynthesis. *S.coelicolor* 2377 *cdaR* mutants showed increased levels of undecylprodigiosin production and noticeably better sporulation than the wild-type. Nuclease protection experiments of the *cdaR* deletion mutants indicate that CdaR positively regulates its own transcription. Multiple copies of *cdaR* do not significantly enhance CDA production or *cdaR* transcription in *S.coelicolor* MT1110. Multiple copies of *cdaR* reduce actinorhodin production and sporulation.

THURSDAY 13 APRIL 2000

0910 Subtyping of bacteria to aid epidemiologic investigations and surveillance: past, present and the future

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CDC's PulseNet, an inter-laboratory pulsed-field gel electrophoresis (PFGE) network, attempts to address the lack of standardization for molecular typing of foodborne pathogenic bacteria and facilitates rapid and reliable intra- and inter-laboratory comparison of DNA macrorestriction patterns. Today, PulseNet is a network of more than 40 public health and food regulatory laboratories in the United States. PulseNet has made it possible to identify disease clusters, particularly those that are diffuse and geographically separated, that are not detected by other means, and to quickly identify the food source of outbreaks. Despite its advantages, subtyping based on PFGE is very difficult because it required rigorous attention to experimental detail and labor-intensive manipulation of data in order to be able to share data between laboratories. As automated DNA sequencing is rapidly transitioning from a research tool to routine use in non-research settings, it is now feasible to consider the application of this technology to the molecular epidemiologic characterization of bacterial isolates. DNA sequencing results are extremely accurate, simplifying lab-to-lab comparisons, have the potential to be more discriminating than traditional assays, and provide precise information on strain relatedness that is only suggested by fragment-based methods.

0950 The rapid characterisation of microbial pathogens using hyperspectral, whole organism fingerprinting and chemometrics

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Recent advances in analytical instruments have allowed the characterisation of microbes from their phenotypic makeup. However, these whole organism 'fingerprinting' techniques tend to produce vast amounts of multivariate data that can be extremely hard to interpret. There is therefore a need to exploit modern statistical and neural computational methods to facilitate automatic microbial identification. Over the last few years the availability of powerful desktop PCs in conjunction with the development of several user-friendly packages which can simulate, for example, artificial neural networks has led to these 'intelligent systems' increasingly being adopted by the microbial taxonomist for pattern recognition.

The rapid accurate characterisation and identification of clinical microorganisms by whole organism fingerprinting will be reviewed, with particular reference to *Candida*, urinary tract infection and the *Bacillus* sporulation biomarker dipicolinic acid. The modern analytical physico-chemical methods discussed will include mass spectrometry (pyrolysis and electrospray ionization) and vibrational spectroscopies (viz. infrared and Raman). The chemometric data processing will concentrate on multivariate statistical analyses, neural networks and evolutionary computing.

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A worldwide survey of *Candida albicans* was conducted using at least 4 microsatellite loci with 9-46 alleles per locus to test for population substructure and levels of gene flow between populations. The high heterozygosity of the microsatellite loci ranging from 0.53 to .74 gave us unusual sensitivity to detect population substructuring. Over 300 isolates were scored for their multi-locus microsatellite genotype. Substantial departures from Hardy-Winberg Equilibrium at two of the microsatellite loci were consistent with the population engaging in some clonal reproduction, but there was little evidence for linkage disequilibrium between pairs of microsatellite loci. We found that about 6% of the total genetic diversity was distributed between populations with $F_{st}=0.06$, suggesting some mild population substructuring. This estimate of F_{st} yielded an estimate of gene flow of about 7 migrants per generation. There was also some evidence for isolation by distance with gene flow following off with geographic distance between sites sampled.

1140 Viral population genetics and the evolution of drug resistance in HIV

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HIV infection is characterised by rapid expansion from a small to a very large population, which maintains a very high replication rate for many years. Turnover of viral genomes is very rapid, with the half life of free virus as low as 3 hours and that of productively infected cells about 1.5 days. In early infection, variability is very low but the high rate of turnover and the high mutation rate of the virus combine to generate genetic variability later in infection.

Despite the very large population size, variation at synonymous sites remains restricted even after many years. Detailed analysis of the pattern of genetic variability in infected patients indicates a population which is evolving as if it was much smaller than it really is. This is also seen in the evolution of drug resistance: single mutations with large effect rapidly take over the population, but mutations with lesser effect appear much more slowly, or not at all, as would only be expected in a relatively small population where chance, or stochastic, effects are important.

Several factors may be responsible for stochastic effects in HIV evolution. The viral population goes through a major bottleneck when a new infection is founded, productive replication occurs in small distinct cell aggregations in solid lymphoid tissue leading to many small subpopulations, and it is continually responding to fluctuating selective forces arising from host immune responses. The clinical significance lies in the unpredictability associated with evolution of small populations especially for the evolution of complex genotypes in response to combination therapy. More detailed understanding of these processes will be important for a clearer understanding of the evolution of drug resistance in this virus.

1400 The fluorescent AFLP approach to the subspecific classification and molecular epidemiology of bacterial pathogens

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Amplified fragment length polymorphism, AFLP, is an analytic technique that selectively amplifies by PCR a subset of restriction fragments of a bacterial genome. Six and four

primer is fluorophore-labelled, and labelled fragments seen by the laser of an automated DNA sequencer are precisely sized [\pm 1bp]. A clone-specific DNA fingerprint is thereby generated with high discriminatory power. We describe empirically-derived FAFLP analyses, for the purposes of molecular epidemiology, of the bacterial pathogens *Streptococcus pyogenes*, *Staphylococcus aureus* and *Salmonella enteritidis*.

Complete genome sequences are available for certain bacterial pathogens. We have utilized these to predict DNA fragments generated by FAFLP. This permits genotyping where the most informative number of fragments, optimally distributed on a sequencing gel, can be designed, experimentally validated and referenced to the published genome sequence of a reference strain. For *Escherichia coli* we have established that this approach also has phylogenetic significance, by analysis of a genetically diverse reference collection (ECOR) defined by multilocus enzyme electrophoresis. We have carried out predictive FAFLP analyses to investigate epidemiological clonality in *E. coli*, *Mycobacterium tuberculosis* and *Neisseria meningitidis*.

1440 Molecular epidemiology and the subspecific classification of trypanosomes and other parasitic protozoa

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Three subspecies of *Trypanosoma brucei*, the causative agent of human sleeping sickness, are currently recognised. *T. b. gambiense* and *T. b. rhodesiense* are human infective and found, respectively, in West and East Africa while *T. b. brucei* is not infective to humans. Molecular studies using RFLP analysis of repetitive sequences show that a more complex taxonomic situation exists and suggests that different human sleeping sickness foci are associated with different strains of *T. brucei*. Furthermore, these methods can be used for identifying animal reservoir hosts and distinguishing human infective from non-human-infective trypanosomes. Due to considerable difficulties in obtaining infected material in large quantity we are currently improving upon this RFLP/Southern blotting technique by developing a PCR based technique. We have developed a novel method, using positional variability in mobile genetic elements, to detect intraspecific strain variation. Here we report the preliminary results of our studies using this method for the identification of strains of *Trypanosoma brucei* and another protozoan parasite, *Toxoplasma gondii*.

1540 Burkholderia cepacia biopesticides and cystic fibrosis lung disease. Friend and foe

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Previously best known as a nutritionally versatile saprophyte and plant pathogen, *Burkholderia cepacia* has emerged as a cause of life-threatening pulmonary infections in immunocompromised humans, in particular patients with chronic granulomatous disease or cystic fibrosis. *B. cepacia* presents three serious clinical problems: Inherent resistance to antibiotics, potential for epidemic spread and the risk of 'cepacia syndrome', a fatal pneumonia sometimes accompanied by septicaemia. Ironically, the organism also provides agricultural and ecological benefits as a potent biopesticide against fungal crop disease and as a bioremediator of contaminated soils and aquifers. Regulatory agencies in North America are presently faced with Solomon-like judgements (<http://www.epa.gov/scipoly/sap1999>) to balance the biological benefits and infectious hazards of these unusual procaryotes which possess multiple chromosomes and a genomic content twice that of *Escherichia coli*.

referred to collectively as the *B. cepacia* complex. All members of the complex have been cultured from clinical specimens. Unfortunately, advances in the molecular epidemiology of *B. cepacia* infections have not been matched by clarification of the complex bacterial/host interactions responsible for lung disease. Nor, in relation to the task of regulatory agencies, have virulence factors been identified which would allow differentiation of environmental *B. cepacia* from potential human pathogens. Data will be presented to support the hypothesis that there are no scientific grounds to differentiate environmental and clinical strains and that the source of isolation cannot be used to assess the safety of biopesticides, or other preparations, containing members of the *B. cepacia* complex. These issues present a challenging 'test case' for regulatory agencies and a stimulus for collaboration amongst a broad microbiological community.

1620 Unity and diversity of influenza viruses

M. ZAMBON

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Abstract not received

FRIDAY 14 APRIL 2000

0910 Molecular epidemiology and subspecific classification of *Cryptococcus neoformans*

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Cryptococcus neoformans (Sanfelice) Vuillemin is a pathogenic basidiomycetous yeast, which can cause serious infections, especially in immuno-compromised patients. Estimates on the incidence rate in AIDS patients range from 5 to 30%, with the highest rates occurring in sub-Saharan Africa. Although sexual states can be obtained in the laboratory, they have not been observed in nature, and most authors consider the natural mode of reproduction of the pathogen to be clonal.

The world population of *C. neoformans* is dominated by serotype A/MAT⁻ isolates, which are the main source of AIDS-related infection. Our RAPD- and AFLP markers, and IGS sequences suggest a considerable genetic distance between the serotypes. The presence of hybrid AFLP-patterns strongly suggests the occurrence of recombination between the two AFLP-genotypes occurring in *C. neoformans*. We hypothesize that these hybrids originated from matings between isolates with serotype A/MAT⁻ and serotype D/MAT^a. These hybridization events in *C. neoformans*, so far mainly observed to occur in Europe, may explain the relatively high number of serotype D isolates involved in AIDS-related infections on this continent, as virulence genes can be transferred from serotype A to serotype D strains. The presence of hybridization may also explain the karyotype paradox in the species.

The second species, *Cryptococcus bacillisporus*, occurs mainly in the tropics, and is usually not found in AIDS patients. It has a free-living state associated with *Eucalyptus* trees. RAPD- and AFLP studies showed that the main distinction between the populations followed geographical rather than serotype borders. The American (A) genotype, with both serotypes B and C, differs from the Austral/Asian/African (AAA) one, with only serotype B. As in *C. neoformans*, the presence of hybrid AFLP patterns reveals the occurrence of sexual hybridization between the AAA and A genotypes. We hypothesize that the hybridization, so far observed only in the Americas, may be a consequence of the

0950 Quasispecies dynamics and fitness variations in RNA viruses

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Fitness is an important parameter that measures the degree of adaptation (capacity to produce generations of infectious progeny) of a virus to a given environment. Studies with the RNA viruses vesicular stomatitis virus, foot-and-mouth disease virus (FMDV) and human immunodeficiency virus type 1 (HIV-1) have shown that fitness variations can be rapid, intense, and mediated by limited numbers of genetic variations in the viral genome. Recent results will be summarized which suggest that: (i) there are multiple molecular pathways for fitness loss and fitness gain of viral clones replicating in a constant environment; (ii) a virus may show considerable resistance to extinction in cell culture in spite of accumulation of mutations prompted by repeated bottleneck events (plaque-to-plaque transfers leading to an accentuation of Muller's ratchet); and (iii) viral quasispecies are endowed with a molecular memory in the form of minority components of their mutant spectra. These results may be relevant to the interpretation of evolution of pathogenic viruses *in vivo*, in particular the evolution of hepatitis C virus and HIV-1 quasispecies in infected individuals.

1100 Molecular epidemiology and subspecific classification of *Plasmodium falciparum*

K. DAY

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Infectious Disease

Abstract not received

1140 Reconstructing the evolutionary history of HCV and HGV/GBV-C: implications for current epidemiological studies

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The recently discovered hepatitis G virus (HGV) or GB virus C (GBV-C) is widely distributed in human populations, and homologues such as HGV/GBV-C_{CPZ} and GBV-A are found in a variety of different primate species. Both epidemiological and phylogenetic analyses support the hypothesis that GB viruses co-evolved with their primate hosts, although their degree of sequence similarity appears incompatible with the rapid rate of sequence change of HGV/GBV-C over short observation periods. We have obtained evidence that RNA folding from extensive internal base-pairing of the single stranded genome places major constraints on sequence change of these viruses. Although the functional roles of the predicted secondary structures in the coding region of the genome remain unclear, these restrictions may lead to differences in net rate of accumulation of nucleotide substitutions at different sites. The resulting disparity between short and long-term rates of sequence change of HGV/GBV-C violates the assumptions of the "molecular clock", and places a major restriction on the use of nucleotide or amino acid sequence information to reconstruct the evolutionary history of HGV/GBV-C or other viruses evolving under the same structural constraints. We have recently obtained evidence that the evolution of HCV may be influenced similarly by RNA secondary structure, and has led us to a re-evaluation of the evolutionary and transmission history underlying its current global distribution.

1400 Molecular epidemiology and sub-specific classification of mycobacterium tuberculosis

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RFLP analysis using the insertion sequence IS6110, the direct repeat (DR) locus, and the polymorphic GC rich sequence (PGRS) has proven useful for identifying related strains of *M. tuberculosis* (MTB) in epidemiologic investigations. We have recently encountered a strain of MTB that is widespread in the US causing large outbreaks of tuberculosis (TB) in several states. Initially, isolates from 162/191 (90%) patients with culture proven TB who lived in central Los Angeles, CA, 03/94-05/96, were analyzed. Patients whose isolates had identical or closely related IS6110/PGRS RFLP patterns were considered a cluster. Isolates from 96 patients (59%) were clustered in 8 clusters, each consisting of 2-43 patients. Homelessness was found to be a predictor of clustering. For the largest cluster (cluster 210), more patients spent time at 3 homeless shelters compared to patients with unique isolates. Since 1992, the MTB genotyping laboratory in Little Rock has analyzed 4,247 isolates from 13 states. The 210 strain is widespread in the US accounting for clusters in 7 of 13 states. In Texas it has accounted for 151 cases of TB since 1993. To determine whether strain 210's ability to spread was correlated with increased rate of growth, its capacity to grow in human macrophages was evaluated. Strain 210 isolates from the original Los Angeles study grew significantly more rapidly than did isolates from other clusters or isolates that caused disease in single patients who had spent substantial amounts of time in homeless shelters that were sites of TB transmission during periods when these patients were infectious.

1520 Population structure of *Porphyromonas gingivalis* based on sequence analysis of house keeping genes combined with investigation of pathogenicity-associated genes

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P. gingivalis (*P.g.*) has been associated with rapidly progressive periodontitis in numerous studies and is very seldom found in healthy gingival sites. *P.g.* has a considerable pathogenic potential when tested in the experimental animal infection model and it possesses a number of recognized virulence factors including a capsule and highly proteolytic enzymes attacking both immunoglobulins, complement factors, iron-containing plasma proteins etc.

In two recently published phylogenetic studies in *P.g.* based on multilocus enzyme electrophoresis and AP-PCR no clear association between one or a few genotypes and periodontitis was established and the question of inter strain recombination was not clarified. (Loos *et al.*, 1993; Menard & Mouton, 1995). It is however clear, that differences in pathogenic potential among *P.g.* strains do exist and this has previously been linked to differences in capsular structure and proteolytic activity.

Based on multilocus sequence analysis of 5 house keeping genes in 50 strains selected among out collection of *P.g.* strains it is our intention to clarify whether *P.g.* has a clonal populations structure or whether inter strain recombination occurs to any appreciable extent. This will be combined with analyses of virulence associated genes including the recently published pathogenicity island in *P.g.* that seems to have acquired via horizontal gene transfer (Hanley *et al.*, 1999).

At present two gene fragments have been sequenced of the 50 strains and the third gene is in progress and before Christmas we will have the results on the occurrence of the pathogenicity island.

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Between 1996 and 1999 several cases of HIV infection were detected in Doncaster (popn. ~250,000). These cases were linked by a complex network of shared sexual histories in which the only known risk factor was heterosexual intercourse. A series of frozen blood samples were collected in 1998-9 and amplified by PCR to generate full-length gp120 clones. Sequencing demonstrated that all of the transmission events in this heterosexual group involved the B subtype of HIV-1. When relationships between the 13 samples were assessed it became clear that these cases represent at least three separate introductions of HIV-1 into this community. Eleven of the 13 cases were linked, 10 of these cases formed two groups containing closely related viruses. One group contained five patients whose general health was good and who were not receiving HAART, all had a viral envelope sequence which was predicted to utilise the CCR-5 co-receptor (R5). In contrast, a second group of five patients, including the putative index case from which these infections arose, contained only viruses predicted to utilise the CXCR-4 (X4) co-receptor, all patients in this group were unwell (AIDS) and receiving HAART. Some of the cases which were linked by genetic criteria were not linked by contact tracing, implying that further, undiagnosed, cases may exist.

CV02 Characterisation of rotavirus G9 strains isolated in the UK between 1995 and 1998

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G9P[6] and G9P[8] rotavirus strains were identified during 1995/96 through the surveillance of rotavirus strains circulating in the U.K. between 1995 and 1998. An increase in the incidence and spread of sporadic infections with rotavirus genotype G9P[8] across the U.K. was detected in the two following seasons. Partial sequencing of the VP7 gene showed that all the UK strains shared a high degree of homology and were very closely related to G9 strains from the US and from symptomatic infections in India ($\geq 96\%$ homology). The UK strains were more distantly related to the apathogenic Indian strain 116E (85%-87.8% homology). Phylogenetic analysis revealed clustering of the UK strains into 3 different lineages (I to III) and into two sub-lineages within lineage I. There were correlations between VP7 sequence clustering, the P type and the geographical origin of the G9 strains. Partial sequencing of the VP4 gene showed a high degree of homology ($>98\%$) among all the P[6] strains, and the sequences obtained from the P[8] strains clustered into 2 of the 3 global lineages described for P[8] strains associated with other G types. These data may suggest that G9 strains may be a recent importation into the UK, and that the G9P[8] strains may have emerged through reassortment in humans between recently introduced G9P[6] strains and the more prevalent cocirculating G1, G3 and G4 strains which normally carry VP4 genes of P[8] type.

CV03 The detection of enterotoxins and toxic shock syndrome toxin genes in *Staphylococcus aureus* by polymerase chain reaction

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A simple PCR based procedure was developed for the detection of fragments of enterotoxins SEA, SEB, SEC, SED, SEE, SEG, SEH and SEI together with the toxic shock syndrome toxin (TSST-1) genes of *Staphylococcus aureus*. One hundred and twenty-nine cultures of *S.aureus* were selected, 39 of which were recovered from 38 suspected staphylococcal food poisoning incidents. The method was reproducible and 32 different toxin genotypes were recognised. The presence of SE genes were associated with *S.aureus* strains reacting with phages in group III, and the TSST-1 gene with phages in group I. There was a 96% agreement between the PCR results for detection of SEA-D and TSST-1 as compared with a commercial reverse passive latex agglutination assay for the detection of SEs from cultures grown *in vitro*. Enterotoxin gene fragments were detected in *S.aureus* cultures recovered from 32 of the 38 suspected staphylococcal food poisoning incidents, and of these, 17 were associated with SEE, SEG, SEH, and SEI in the absence of SEA-D. These results suggest that the relative importance of different staphylococcal enterotoxins as a cause of food poisoning should be re-examined. In addition these studies should be extended to include the recently described SEJ and further undescribed enterotoxins.

CV04 Molecular epidemiology of *Helicobacter pylori* in England (Mid-Essex): variation in genes associated with vacuolating cytotoxin activity in relation to patient disease severity and age

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Background - The vacuolating cytotoxin (VacA) of *Helicobacter pylori* - a bacterium widely associated with increased risk for peptic ulcer disease, atrophic gastritis and gastric cancer - is a key pathogenicity factor encoded for by the *vacA* gene for which at least nine allelic variants have been identified with a global distribution and different frequencies according to ethnic origin and disease severity. As there is a paucity of information on the epidemiology of *vacA* forms in the British population, we have investigated *vacA* variation in *H. pylori* from dyspeptics in mid-Essex in relation to disease severity and age.

Methods - *H. pylori* were isolated over a four year period from gastric biopsies of one hundred and eighty patients aged 16 to 85 years. Patients were grouped according to the severity of pathology described at endoscopy as peptic ulcer disease (PUD) group, nonulcer dyspepsia with gastritis only (NUD-GAS) group, NUD with oesophagitis (NUD-OES), and NUD with normal mucosa (NUD-NOR) group. Two patients with gastric neoplasia were also included. Isolates were characterised by *vacA* signal/mid-region allele genotyping, and by *vacA* sequence-linked PCR-restriction fragment length polymorphism analysis (SL-RFLP) with *HaeIII* digestion.

Results - Prevalence rates for the *H. pylori vacA* genotypes were 40% for s1/m1, 45% for s1/m2, and 12% for s2/m2. *HaeIII* RFLP analysis of *vacA* showed the m1 and m2 alleles were diverse with 86 different profiles amongst 137 strains. Variation amongst 41% (56/137) of strains was accounted for by eight predominant *vacA* RFLP profiles with the two m-forms each represented by a characteristic and distinct family

RFLP profiles of five strains with mixed m1+m2 forms matched the m2 family. The various *vacA* forms were widely distributed in *H. pylori* from the patients of different gastric pathologies and ages.

Conclusions – PCR-RFLP analysis of *H. pylori vacA* provided more precise information than *vacA* sm genotyping on the location of *vacA* intragenic variation, and demonstrated a high degree of interstrain diversity. Certain RFLP profiles were closely associated with either the mid region m1 or m2 allele, and provided evidence of two distinct families of *vacA* variants, that were widely distributed throughout the population of British dyspeptics investigated. Prevalence of a particular *vacA* form was mostly independent of disease severity and patient age, and did not explain variations in disease occurrence but might provide the basis in combination with other markers for stratifying disease risk in the future.

CV05 Hierarchical amplified fragment length polymorphism technique for *Salmonella* epidemiology. T. PETERS & E.J. THRELFALL

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Amplified fragment length polymorphism (AFLP) is a PCR-based DNA fingerprinting technique whereby restriction fragments may be visualized without prior knowledge of nucleotide sequences. In AFLP analysis, bacterial genomic DNA is digested with a restriction enzyme and ligated to adapter oligonucleotides. A subset of DNA fragments are then amplified using primers which contain adapter-defined sequences. The three basic stages of AFLP analysis are: i) digestion of genomic DNA by restriction enzymes ii) ligation of oligonucleotide adapters to restriction fragments, and iii) amplification of a subset of ligated fragments by PCR.

Selective amplification is achieved by the use of primers containing adapter-defined sequences with one additional arbitrary nucleotide. We used four primers complementary to the adapter sequence but each differing in the final 3' base that extended into the fragment DNA. The usefulness of these primers for fingerprinting *Salmonella enterica* was assessed in a hierarchical manner, looking for banding patterns that would distinguish between strains without being too complex for comparative analysis.

Using a single-enzyme approach we have used this method to fingerprint 30 strains of *S. enterica* belonging to 14 different serotypes. AFLP profiles derived from *Hind*III fragments differentiated between the serotypes. In addition, AFLP profiles for each serotype differentiated between the phage types and individual strains. The technique is relatively rapid and has given reproducible and discriminatory results. This hierarchical AFLP technique may provide a valuable addition to existing methods for the DNA fingerprinting of *S. enterica* for epidemiological studies.

CV06 A single-tube real-time nested pcr for detecting human papillomaviruses

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A real-time PCR (LightCycler) for detecting human papillomaviruses (HPV) of different types with the same sensitivity as a commonly used nested PCR on the block-based thermocycler was developed.

The MY09/MY11 (degenerate) and GP5+/GP6+ (consensus) oligonucleotide primers, detecting a broad range of HPVs, were used on the LightCycler. The marked differences of optimal reaction conditions of the first round and second round PCRs, and the use of anti-*Taq* antibody have allowed sequential amplification in a closed single tube PCR reaction, including the detection of the PCR amplicons

conventional nested PCR, using the same primer pairs and 20 HPV types. Twenty input copies of HPV 11 and 16 DNA were detected by both methods. HPV types 2, 3, 5-8, 10, 14, 17, 18, 20, 31, 33, 49, 50 and 57 could also be detected by both PCR methods. The PCR on the LightCycler was performed in 90 minutes, whereas the conventional PCR, including amplicon detection by gel electrophoresis, took more than 10.5 hours. This permits a greater throughput of samples and reduces the risk of contamination often associated with nested PCR.

CV07 Variation in antibiotic resistance genes amongst multiresistant *Salmonella enterica* serotype Typhimurium phage type U302 (MR U302) isolated from humans, animals and foods

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Multiresistant *Salmonella enterica* serotype Typhimurium definitive phage type 104 (MR DT 104), commonly resistant to ampicillin, chloramphenicol, streptomycin, sulphonamides and tetracyclines, has emerged in recent years as a worldwide health problem in humans and food animals. In this study forty multiresistant *S. Typhimurium* phage type U302 strains (MR U302), from humans, animals and foods and closely related in terms of phage type reactions and resistance spectrum to the MR DT 104 phage type, were examined to determine whether they possessed the same chromosomally mediated resistance genes that are present in MR DT 104. PCR amplification of *bla*_{CARB-2} (ampicillin resistance), *cmlA* (chloramphenicol resistance), *aadA2* (streptomycin resistance), *sul1* (sulphonamide resistance) and *tetA* -class G (tetracycline resistance) was applied to establish the prevalence of these resistance genes. In addition *bla*_{TEM} primers were employed for the detection of sequences encoding TEM-type β -lactamases. Conjugation experiments were carried out on *bla*_{TEM} positive strains to determine whether ampicillin resistance could be transferred. Plasmid profiling and PFGE were performed to differentiate the MR U302 strains and to provide a molecular comparison with MR DT 104.

34/40 strains were positive for *bla*_{CARB-2}, *cmlA*, *aadA2*, *sul1* and *tetA* (G); one strain was also positive for *bla*_{TEM}. Four strains were positive for *bla*_{TEM}, *aadA2* and *sul1*, one strain was positive for *aadA2* and *sul1* and one strain for *bla*_{TEM} only. *bla*_{TEM}-mediated ampicillin resistance was transferred to *E. coli* K12 from four MR U302 isolates. Strains carried up to six plasmids and thirty-five different plasmid profiles were identified; a 60 MDa plasmid was the most frequently identified (35/40 strains). Nine different patterns were generated by PFGE, although 31/40 strains had a PFGE profile identical to the XTm1 pattern predominant in MR DT 104.

The results show that although many of the MR U302 strains studied possessed the same antibiotic resistance genes as MR DT 104 some have acquired different mechanisms of resistance to ampicillin, chloramphenicol, streptomycin, sulphonamides and tetracyclines compared to MR DT 104. These may be borne on transmissible plasmids. Variation at the genotypic level has been further demonstrated by PFGE analysis. Plasmid profiling has proved to be particularly effective in sub-dividing the MR U302 strains and in contrast to MR DT 104, no single plasmid type predominated.

CV08 Cloning and expression of the Ebola (Zaire) virus nucleocapsid protein: evaluation as a diagnostic reagent for Filovirus infections

JANE BOWEN, ANTHONY P. FOOKS, GRAHAM LLOYD

The filoviruses Marburg and Ebola cause severe, often fatal haemorrhagic disease in humans and primates and are among the most pathogenic of human viruses. The viruses have been inadvertently imported into Europe and the U.S. in shipments of non-human primates from Africa and Asia on several occasions. Our aim was to develop rapid, sensitive and robust diagnostic assays for the diagnosis of potential filovirus infections in imported non-human primates without the use of infectious material requiring high containment. We have cloned and expressed the nucleocapsid protein (NP) of Ebola virus (EV) Zaire strain, with an N-terminal histidine tag under the control of the polyhedrin promoter in a baculovirus vector. Analysis of infected insect cells by immunofluorescence, PAGE and immuno-blotting identified a specific 104kDa protein which was similar in size to that produced from EV-infected Vero cells. Infected insect cells produce high levels of recombinant EV NP which was purified using affinity resins. The performance of the recombinant NP protein was assessed in indirect antibody ELISAs with panels of non-human primate and human convalescent sera. The assay was specific for the detection of serum IgG from both African and Asian subtypes of Ebola virus infection. In addition, the assay was sensitive and can be performed in a normal diagnostic laboratory in under 6 h. This assay should form the basis of a diagnostic assay for filovirus infections in both humans and imported non-human primates entering the UK.

CV09 Antigenic studies of SIV nef clones expressing wild type and attenuated phenotypes in macaques

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The *nef* gene of primate lentiviruses codes for a regulatory protein which has been demonstrated to play an important role in augmenting viral growth *in vivo*. At NIBSC we are studying the structural, molecular and biological properties of SIV Nef variants *in vitro* and *in vivo*.

We have prepared a panel of monoclonal antibodies which bind specifically to SIV Nef. Selected antibodies from this panel display differential binding to the Nef protein derived from attenuated C8 and wild type J5 variants of SIV that differ only through selective sequence changes in the *nef* gene (Arnold et al, 1999, AIDS. Res. Hum. Ret; 15 (12), 1087-1097).

Eight SIV *nef* variants were cloned into the bacterial expression vector pGEX-4T3 to generate recombinant GST-Nef fusion proteins. By using Glutathione coated plates we have developed a single step method to purify the fusion protein and evaluate the binding of SIV Nef fusion proteins by the specific anti-Nef monoclonal antibodies. We confirmed the value of the method by comparing the results of this ELISA based assay with FACSCAN analysis of recombinant SIV expressing identical Nef variants. We have now gone on to characterise the antigenic properties of SIV *nef* variants recovered by Whatmore et al (J. Virol. Aug 69(8); 5117-23) from macaque 45R infected with attenuated SIVmacC8 but which reverted *in vivo* and repaired the attenuating 4 amino acid deletion in the *nef* gene. The results of these studies will be presented and interpreted in terms of the predicted structure of the SIV Nef protein.

CV10 High incidence of multiple genotypes from patients infected with hepatitis C virus in the South West of England

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Human sera which tested positive for Hepatitis C virus (HCV) RNA by PCR were genotyped using two commercially available reverse hybridisation assays (Inno-LiPA HCVII (Innogenetics, Zwijndrecht, Belgium) and HCV-RNA Genotyping kit (Sorin, Biomedica, Saluggia, Italy)) and by sequence analysis of diagnostic regions of the 5' non-coding region (5'NCR) and core and non-structural 5 (NS5) genes. Whilst RT-PCR products were generated from all serum samples using all primer sets directed to the 5'NCR and core region, 12 samples could not be amplified using primers targeted at the NS5 region. Stable clusters were identified using core and NS5 sequence data at the subtype level, but only at the type level for 5'NCR.

A consensus genotype was assigned to 21 of the 48 serum samples using all methods. Five samples were 1a, six were 1b, one was 2b and nine were genotype 3a. PCR products generated from hepatitis RNA hybridised to multiple genospecific probes using Innogenetics (12 samples) and Sorin (13 samples) genotyping kits. Two samples failed to hybridise significantly to any of the genospecific probes using the Sorin assay and another sample failed using the Innogenetics assay.

A surprisingly large number of sera were ascribed multiple

POSTERS: Systematics & Evolution Group

SE01 Characterisation of enterovirus isolates from patients with heart muscle disease in selenium-deficient area of China

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In order to understand molecular epidemiology of enterovirus infection in heart muscle disease occurring in selenium-deficient area of China, 6 enteroviruses (2 with unknown serotype), isolated from heart tissue, blood or faecal samples of patients with endemic cardiomyopathy (Keshan Disease) or during outbreak of myocarditis, were evaluated by DNA sequence analysis of RT-PCR products. The pathogenesis of these isolates were tested in adult and suckling mice. Sequence data from the 5'-nontranslated region of these viruses confirm that they belong to coxsackie B virus (CVB) phylogenetic cluster. Sequence analysis of the VP1 coding region shows that two isolates have highest homology with CVB2 and remaining 4 isolates including the two unknown serotypes are most close to CVB6. Nucleotide sequences among these viruses vary, indicating no contamination. None of these viruses caused pathology in the heart of MF1 mice, although pancreatitis was evident. However three CVB6-like viruses caused death in suckling mice, similar to a virulent CVB3 laboratory strain. The sequence data confirms that CVB genotypes are predominant in endemic region of Keshan Disease. Genotyping of enterovirus is essential, as neutralisation assay may fail to type the virus. Animal experiments indicate that different pathogenesis may exist among these isolates.

SE02 Evidence for a genetically stable clone of *Campylobacter jejuni*

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The genetic stability of selected epidemiologically-linked strains of *C. jejuni* during outbreak situations was investigated using AFLP. Strains isolated in 1998 were investigated from chickens and the environment around three broiler houses which were geographically related. There was little similarity in strains between outbreaks. However, the strains from within all three chicken outbreaks, including strains isolated from the house floor, feed lines, and chicken faeces, were identical at over the 95% level of similarity confirming the genetic stability of these strains within the short time courses of chicken flock outbreaks. Strains were also investigated from a human outbreak, in 1981, thought to be due to contaminated water (Palmer *et al.*, 1983). Three AFLP profiles were recognised from this outbreak confirming the serotyping undertaken at that time. The major type (serotype P6:L6) isolated from this outbreak was exemplified by strain 81116. This isolate is a well-characterised laboratory strain. Nevertheless the AFLP profile of strain 81116 was identical with all the other P6:L6 strains from the outbreak indicating that it has remained remarkably stable over almost 20 years despite being subtyped on many occasions. Interestingly the AFLP profiles of the P6:L6 strains from the human outbreak and the strains from one of the chicken outbreaks were identical at the 94% level. This similarity is also remarkable and suggests that some clones of *C. jejuni* remain genetically stable in completely different environments over extremely long periods of time and considerable geographical distances.

SE03 A study of the population structure of *Campylobacter jejuni* using multi-locus sequence typing (MLST)

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Campylobacter jejuni is a major cause of human enteritis in the world today. The main source of human infection is believed to be the consumption of contaminated poultry meat, water and milk. Recent epidemiological data suggests

of *C. jejuni*, using multi-locus enzyme electrophoresis (MLEE), has indicated that the population may be at linkage equilibrium whilst in another some host-specific association with certain clones and also an association between host source and certain enzyme alleles has been observed. In this study we have adopted a DNA-based version of MLEE, multi-locus sequence typing (MLST) as a method to study the population structure of *C. jejuni* strains isolated from a wide variety of sources. Here, we discuss the choice of loci chosen for this analysis and present some preliminary MLST data obtained from an epidemiologically well-characterised strain set isolated from various sources within Europe.

SE04 AFLP-genotyping of *Campylobacter fetus*
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Campylobacter fetus is divided into two subspecies fetus and venerealis. The high homology at genetic level is in contrast with significant differences in clinical presentation. The genitally transmitted subsp. venerealis is the cause of abortion and infertility in cattle. Subsp. fetus can cause abortion in sheep after oral infection. Subspeciation is important for both clinical and economical reasons. Statutory import requirements for semen and embryos for some countries specify freedom either from both subspecies or from subsp. venerealis only. However phenotypic subtyping of *C. fetus* is difficult and can give ambiguous results. Recently AFLP has been successfully applied to the molecular typing of *Campylobacter*s. Therefore AFLP was performed on 71 *C. fetus* clinical isolates from different geographical locations and hosts. As expected, the genetic relationships between the different isolates were high. However, in general, patterns differentiating the subspecies were observed. Thus AFLP-subtyping seems to be a suitable method for subspeciation. Nevertheless some strains showed anomalous patterns, inconsistent with phenotyping and PCR-based genotyping results. Whether these results reflect different genetic lineages is now under investigation. The value of phenotyping, PCR-genotyping and AFLP for subspecies differentiation will be reported.

SE05 AFLP fingerprinting of *C. jejuni* from chickens and from patients with gastroenteritis or Guillain-Barré or Miller Fisher syndrome
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C. jejuni is the most common cause of foodborne bacterial gastroenteritis. Serious sequela of *C. jejuni* infections are the neurological disorders Guillain-Barré (GBS) and Miller Fisher syndrome (MFS). A major route for infection is consumption of contaminated poultry products. In this study the high-resolution typing method Amplified Fragment Length Polymorphism analysis (AFLP) was used to study genetic relationships and clonality of *C. jejuni* from human and poultry. AFLP analysis of 125 *C. jejuni* strains using calculated similarities levels between banding patterns based on correlation- and band-based coefficients and UPGMA cluster analysis, identified a highly heterogeneous population of *C. jejuni* infecting chickens and humans in the Netherlands. The strains were separated in two distinct genetic groups with only 40 % homology. Both groups contained smaller clusters with human and chicken strains. Strains

environment, are genetically related to strains causing human diseases. We conclude that the studied *C. jejuni* population is genetically highly heterogeneous and identified no unique AFLP fingerprints of *C. jejuni* strains infecting chickens or those related with human gastroenteritis, GBS or MFS.

SE06 *Fla* typing for thermophilic campylobacters using consensus primer sets
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Campylobacter jejuni and *Campylobacter coli*, are major cause of acute bacterial gastroenteritis throughout the UK and the developed world. In order to understand the epidemiology of these pathogens many typing systems have been devised. Evaluations of genotypic methods have been reported, with flagellin gene profiling emerging as a method suitable for rapidly investigating large numbers of *C. jejuni* and *C. coli*. Several different protocols for PCR/RFLP typing of the *Campylobacter* flagellin gene locus have been developed. However, considerable variation in these procedures exists, particularly in primer design. Although the majority of primer sets have been directed at the *flaA* genes, others have been chosen to amplify *flaA* and *flaB* in combination. Variation in positioning of primers within the flagellin genes can have major consequences on RFLP profiles obtained. To assist in standardisation of flagellin gene typing a PCR protocol was developed for consensus primers, defined as a result of aligning available gene sequences of *flaA* and *flaB*, for separate amplification of each gene. A comparison of the sensitivity of the *flaA* and *flaB* consensus typing scheme with that of commonly applied protocols based on *flaA* and *flaA/B* in combination was made using over 30 well characterised isolates of *C. jejuni* and *C. coli*. All amplicons were digested using *Hin* fl and *Dde* I and both purified DNA and lysed cells were tested as the target for amplification. The results of the evaluation will be presented.

SE07 Rapid identification and typing of methicillin resistant *Staphylococcus aureus* using intact cell matrix assisted laser desorption ionisation-time of flight mass spectrometry (ICMS).

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Methicillin resistant *Staphylococcus aureus* (MRSA) is a major nosocomial pathogen. Rapid differentiation of MRSA from methicillin sensitive *S. aureus* (MSSA) is therefore essential to ensure effective infection control and appropriate therapy. Two strains of epidemic MRSA (bacteriophage EMRSA) types 15 and 16 now predominate in England and Wales necessitating the development of molecular sub-typing methods, including Pulse Field Gel Electrophoresis, to provide improved discrimination. These methods can be time consuming. Rapid methods to detect methicillin resistance based upon *mecA* gene detection have been developed. However, many coagulase negative staphylococci (CNS) are *mecA* positive and some MRSA are phenotypically methicillin sensitive.

Recently, MALDI-TOF-MS has been applied to intact bacterial cells (ICMS), providing a reproducible, spectrum within minutes. Bacterial cells are applied to a sample slide, matrix is added and the slide is transferred to the mass spectrometer. Negative and positive ions are produced and are sequentially delivered to a detector in order of their mass:charge ratio (m:z) and either can be analysed.

A panel of well characterised isolates of MRSA, methicillin sensitive *S. aureus* (MSSA) and coagulase

analysed using two different instruments. Spectra for individual specimens were compiled, averaging results from 100 shots taken across the width of the specimen for m/z values 500 -10,000. Spectra for the five replicates were compared to determine within-isolate peak reproducibility, then summed to minimise random effects of baseline drift and noise. Only peaks with intensities > 0.4mV following base line subtraction were considered in the analysis. The presence and relative intensities of peaks represent fingerprints for the particular isolate. Results were analysed within Excel spreadsheets.

Reproducible spectra were obtained from each of the two instruments and different culture medium. However, the type of culture media did have an effect on the spectrum. However, for organisms grown on Columbia agar and Methicillin Mannitol Salt Agar, it was possible to highlight key areas within the spectrum that were relevant for species identification; differentiation of MRSA from MSSA; and subtyping, similar to that obtained with PFGE.

These results show the enormous potential of ICMS to rapidly (within minutes) identify and subtype MRSA **simultaneously**, directly from colonies on primary culture.

SE08 Rapid identification of *Campylobacter sp* using intact cell matrix assisted laser desorption time of flight mass spectrometry (ICMS)

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In the UK, the most common cause of bacterial gastroenteritis is *Campylobacter sp*, primarily *C. jejuni*. The epidemiology of this disease, however, is poorly understood due partly to the lack of comprehensive identification within Clinical Diagnostic Laboratories. In addition improved rapid subtyping methods are required to provide improved strain characterisation for epidemiological studies of campylobacter infections.

Recently, MALDI-TOF-MS has been applied to intact bacterial cells (ICMS), providing a reproducible, spectrum within minutes. Bacterial cells are applied to a sample slide, matrix is added and the slide is transferred to the mass spectrometer. Negative and positive ions are produced and are sequentially delivered to a detector in order of their mass:charge ratio (m/z) and either can be analysed.

A diverse set of isolates (n=38) of *Campylobacter sp*, *Helicobacter sp*, and *Arcobacter sp* were analysed with a Kompact MALDI 2 linear, time of flight mass spectrometer (Kratos Analytical) after 24 hrs incubation on Columbia Blood Agar at 37°C in a reduced oxygen atmosphere. Spectra for individual specimens were compiled, averaging results from 100 shots taken across the width of the specimen for m/z values 500 -10,000. Spectra for the five replicates were compared to determine within-isolate peak reproducibility, then summed to minimise random effects of baseline drift and noise. Only peaks with intensities > 0.4mV following base line subtraction were considered in the analysis. The data obtained was transferred to an Excel spreadsheet and analysed.

Campylobacter sp were easily distinguished from *Helicobacter* and *Arcobacter sp*. There were several common peaks (m/z values) within the genus, but there were also areas within the spectrum that allowed species identification. It was possible to identify *C. jejuni*, *C. coli*, and *C. fetus* very rapidly (minutes). At a finer discriminatory level, there were sufficient differences between the spectra of *C. jejuni sp jejuni* (n=18) isolates to show its potential for rapid subtyping.

SE09 Semi-automation of multi-locus sequence typing (MLST) for meningococci

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The Scottish Meningococcus and Pneumococcus Reference Laboratory (SMPRL) provides a national service for the laboratory confirmation of meningococcal and pneumococcal disease in Scotland. Part of this service includes the serogrouping of meningococcal isolates followed by typing and sub-typing. The procedures for this are labour intensive but important for the identification of linked cases and the surveillance of disease so that effective public health measures can be taken. However, epidemic strains of meningococci are now indistinguishable by current methods. The SMPRL have therefore started using multi-locus sequence typing (MLST) for the full identification of meningococcal isolates. MLST produces nucleotide sequence data of 500 base-pair segments from seven house-keeping genes providing results that are digital and therefore highly portable between laboratories. MLST can provide data which is useful for the public health management of clusters or outbreaks in institutions such as schools, and also for general disease surveillance on a national basis thereby providing information to determine national vaccine policy. The SMPRL is using the MWG-Biotech Roboamp-4200, a 96-well format liquid handler, and LICOR L-4200 L2 DNA sequencer to provide MLST as a new reference service for Scotland. This poster describes the methodology for semi-automation of MLST for meningococcal isolates by the SMPRL.

SE10 Distribution of enterotoxin genes in some strains of *Bacillus Thuringiensis*

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Bacillus thuringiensis (Bt) is a Gram-positive, endospore forming bacterium that is used widely for the control of various insect pests and vectors of disease. Insect toxicity is largely derived from the delta-endotoxin, a crystalline protein that accumulates in the cell as a parasporal body. Bt is closely related to *Bacillus cereus* (Bc), a widely distributed bacterium that is found in the soil and on plants. Numerous taxonomic studies, both molecular and phenotypic, indicate that Bc and Bt are members of the same species. Bc causes a diarrhoeal form of food poisoning that arises from ingestion of the bacterium and subsequent colonization of the gut. Two tripartite toxins have been implicated in the syndrome, a haemolytic toxin (Hbl) and a non-haemolytic toxin (Nhe) and single component toxin, BceT. Since many foods, particularly imported vegetables, are treated with Bt, we examined the distribution of these toxin genes in 74 strains of Bt using PCR. Most (97%) possessed nhe and a majority bceT (85%) while Hbl was detected in only 54 strains (73%). We have also characterized our strain collection by Randomly Amplified Polymorphic DNA (RAPDs) but there was limited correlation between strain type and presence of toxin genes.

WEDNESDAY 12 APRIL 2000

0930 The endocytic pathway; a key player in viral replication from entry to assembly

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Enveloped viruses use the membrane systems of the host cell to facilitate their replication. The endocytic pathway is crucial for a number of different viruses, including the human and simian immunodeficiency viruses. For HIV, cell surface expression of the receptors for these viruses, both CD4 and chemokine receptor, is modulated by endocytosis and regulated by both cellular and viral mechanisms. Endocytic down modulation of receptor expression can protect cells from viral infection in tissue culture and can contribute to viral production. The cytoplasmic domain of the viral envelope protein contains multiple sorting signals. These signals operate in both the exocytic and endocytic pathways; they limit envelope expression at the cell surface and cause the protein to be directed to endocytic organelles. The presence of these signals may influence both the location and timing of viral assembly.

The molecular mechanisms and the biological consequences of these regulatory events will be discussed.

0945 Receptor usage and entry of measles virus into cells

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The high morbidity and mortality rates caused by measles are due to a transient immunosuppression and complications following the acute infection. Interaction with measles virus (MV), irrespective of which strain, leads to the induction of a proliferative inhibition of lymphocytes. The observed block in cell cycle progression in cells of haematopoietic origin is caused by a direct interaction of the MV envelope proteins with the target cell surface. CD46 was identified as a receptor for MV-vaccine strains. Such strains and some wild-type strains cause the downregulation of CD46 from the cell surface after infection of cells or contact with MV-envelope proteins. However, most recent wild-type MV-strains, which were isolated using B-cell lines, do not downregulate CD46, and do not use CD46 as receptor. These strains bind with lower affinity to their target cells than CD46-using strains. Recombinant MVs with wild-type haemagglutinin (H) do not interact with CD46, but do induce the proliferative inhibition in lymphocytes. Receptors involved in the attachment of these MV wild-type isolates and/or the proliferative inhibition are subject of investigation.

1100 Infection by influenza viruses

J. SKEHEL

NIMR, London

Abstract not received

1145 Post-receptor events during poliovirus entry into cells

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Cell entry by viruses is a major determinant of host and tissue

solve the topological problem of traversing cellular membranes to deliver the viral genome into the cell cytoplasm. Poliovirus binding to its receptor (PVR) on the cell surface induces a conformational transition which generates an altered particle (the 135S particle). Although the role of the 135S particle is controversial, recent studies strongly indicate that this particle is an intermediate in the poliovirus entry pathway. The studies also demonstrate that binding to PVR provides at least two functions for entry: (1) Docking the virus particle close to the cell surface and (2) Altering the energetics of the virus particle such that 135S particles are formed under physiological conditions. In addition, other studies recently have identified additional stages which occur subsequent to PVR binding. These stages indicate that additional factors are required to trigger delivery of the viral genome into the cytoplasmic compartment of the cell.

1400 The coxsackievirus and adenovirus receptor

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The coxsackievirus and adenovirus receptor (CAR) is a 46 kD cell surface protein that functions as a receptor for coxsackie B viruses and many adenovirus serotypes. Both viruses bind directly to the CAR extracellular region, which is composed of 2 immunoglobulin-like domains. Some coxsackie B viruses also bind to another receptor, CD55; however, although CAR facilitates essential post-attachment events in infection, CD55 does not. Post-attachment events in adenovirus infection involve coreceptors, primarily av integrins.

CAR expression is a major determinant of tropism for both virus groups. On polarized epithelial cells, CAR is restricted to the basolateral surface, and its absence from the luminal surface of airway epithelium has been an obstacle to adenovirus-mediated gene therapy for cystic fibrosis. CAR localization in polarized cells is a function of the cytoplasmic domain, which is highly conserved in several species, but is not essential for virus infection. Retargeting CAR to the luminal surface of polarized cells permits adenovirus-mediated transduction, but reveals the glycocalyx as an additional barrier to gene delivery.

1445 Retargeting and protection of adenovirus from antibody recognition by modification with hydrophilic polymer

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Adenovirus is frequently used in cancer gene therapy strategies, despite several limitations that may restrict its success, for example its wide tissue tropism and the influence of the immune system. We have developed a non-genetic technique for modifying the surface of adenovirus virions using a ligand-bearing polymer in order to alter its interaction with the immune system and its tropism. The polymer reacts with primary amino groups on the virus surface, which not only affects its ability to interact with antibody but also prevents CAR binding, as shown by attachment studies. Biological activity is restored by incorporation of a targeting ligand, basic fibroblast growth factor (bFGF), onto the polymer coat. Targeting was shown

tumour cells was shown to be enhanced using retargeted polymer-modified virus. The effect of polymer coating on antibody recognition was tested in capture ELISA and neutralisation assays. The ELISA showed that the polymer shields surface epitopes on the virus from antibody recognition. Neutralisation assays were carried out in the presence of anti-Ad5 antibody or ascites fluid known to contain anti-adenovirus neutralising antibodies; it was shown that polymer modified bFGF targeted virus was not susceptible to neutralisation at dilutions of ascitic fluid that block infectivity of unmodified virus. Polymer modification of adenovirus is an effective way of altering viral tropism and evading the immune system.

1500 Alternative entry routes for DAF-using enteroviruses

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We have been studying the cellular proteins that enteroviruses use as receptors and how different receptors may result in alternate entry pathways. The work has mainly focused upon an echovirus type 11 isolate, 207, and its derivatives 207r and 207m. 207 infects HT29 (colon carcinoma) and RD (rhabdomyosarcoma) cells using Decay Accelerating Factor (DAF, CD55) as a receptor. 207r and 207m do not use DAF and use as yet unknown receptors.

DAF is a Glycosyl Phosphatidyl Inositol (GPI) linked protein and these proteins have been shown to cluster in detergent insoluble lipid raft domains upon cross-linking. These domains have been shown to be entry routes for other viruses such as SV40.

We have studied the role of these domains in 207 entry using various drugs which specifically disrupted lipid raft function and compared their effect with the non-DAF using viruses 207r and 207m.

1515 Integrin receptors of foot-and-mouth disease virus

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Field isolates of foot-and-mouth disease virus (FMDV) bind to members of the integrin family of integral membrane proteins through a conserved tri-peptide RGD, on capsid protein VP1. To date, $\alpha 3$ is the only RGD-dependent integrin that has been shown to act as a receptor for FMDV, but this integrin has limited expression on epithelial cells and cells of the lymphoid origin and it is these cell types in which FMDV is likely to reside during the initial stages of infection. However, several other RGD-dependent integrins are expressed on these cell types where they may have potential to act as cellular receptors for FMDV. Of these $\alpha 6$ is expressed exclusively on epithelial cells, making this integrin a more likely candidate for use as a receptor by FMDV during the initial phases of infection. In this report we show that human colon carcinoma cells (SW80) that are normally non-permissive for FMDV become susceptible to infection as a result of transfection with the integrin $\alpha 6$ -subunit and expression of $\alpha 6$ at the cell surface. Integrin $\alpha 6$ serves as the major site for virus attachment on the $\alpha 6$ -

virus binding and infection of $\alpha 6$ -transfected cells is mediated through an RGD-dependent interaction that is specifically inhibited by a monoclonal antibody (10D5) that recognizes $\alpha 6$. These studies establish a role for $\alpha 6$ as a cellular receptor for FMDV.

1600 Early stages of influenza virus entry into MV-1 lung cells: involvement of dynamin

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Viruses generally have one of two mechanisms for entry and uncoating. They can enter the cell either by endocytosis or by direct fusion at the plasma membrane. We have established a novel mink lung (Mv-1) cell line that expresses a dominant-interfering form of dynamin-1 (K44A) under the control of a tetracycline-responsive element and studied the early events in influenza infection using these cells. We found that influenza virus binds equally to both induced and un-induced cells, but in K44A-expressing cells electron microscopy showed viruses trapped in deep coated pits and irregular-shaped tubular structures that contain discrete coated regions. We also show by immunofluorescence and confocal microscopy that entry of incoming virus into the nucleus is blocked in K44A-expressing cells. Virus replication was assayed by immunofluorescence microscopy and was strongly inhibited at both early and late times post infection in K44A-expressing cells. Virus infectivity was inhibited by approximately two log units in cells expressing K44A dynamin, when analyzed by influenza plaque assay. Overall, these data show that dynamin is required for efficient influenza virus entry, presumably due to its function in release of vesicles from coated pits.

1615 Determining the importance of human chemokine receptor CXCR4 in MAEDI-VISNA virus entry

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Human CD4 and the chemokine receptor CXCR4 together mediate entry of predominantly T-cell tropic strains of human immunodeficiency virus (HIV)-1. Feline immunodeficiency virus (FIV) and certain strains of HIV-2 have been shown to use human CXCR4 alone for entry. These findings have spurred the notion that CXCR4 might represent a common lentivirus receptor. Maedi-visna virus (MVV) is a lentivirus that infects the macrophage cell lineage of sheep and goats. The identity of the MVV receptor is not known to date. Human U87 cells and U87 cells transfected with human CXCR4 were challenged with MVV. In both cases syncytia formation occurred after 3-4 days compared to the uninfected controls. Infected U87/huCXCR4 cells had larger and more extensive syncytia compared to U87 cells. MVV was also able to infect and induce syncytia formation in human HOS cells which do not express chemokine receptors. These preliminary results suggest that human CXCR4 is not absolutely required for MVV entry, however it is clear that the presence of human CXCR4 on U87 cells augments virus-induced cell fusion.

1630 Heparan sulphate is the primary attachment molecule for respiratory syncytial virus

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Human respiratory syncytial virus (HRSV) adsorption to mutant CHO cells has been shown to be dependent upon the presence of cell surface glycosaminoglycans (GAG's). Flow cytometric investigation of the interaction of HRSV to mutant L cells demonstrated that GAG heparan sulphate (HS) but not chondroitin sulphate was required. Enzymatic removal of HS from cells reduced the adsorption of HRSV to a variety of cell lines whereas treatment of HRSV had no effect suggesting HS on the virus is not required for function. Sulphation levels were important: pure heparin (HS analogue) but not under-sulphated heparin reduced adsorption and infectivity of HRSV. The virus attachment G protein was shown to bind heparin using heparin affinity chromatography and purified G protein adsorbed to cells in a HS dependent manner. Results suggest that HRSV uses HS as a primary attachment molecule and that adsorption involves the G protein. Clinical isolates are being investigated for dependence on HS for adsorption, and for a second high affinity receptor/entry interaction.

1645 Construction and characterization of chimeric E2 glycoproteins of hepatitis C virus: analysis of E2 regions interacting with CD81

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A truncated form of hepatitis C virus (HCV) glycoprotein E2 (E2660) has been shown to bind human CD81, a putative receptor on the target cells. We have compared the ability of two divergent E2660 glycoproteins derived from HCV genotype 1a strains, Glasgow (Gla) and H77c, to bind a panel of conformation-dependent monoclonal antibodies (MAbs) and CD81. In contrast to H77c, Gla E2660 failed to react with conformation-dependent MAbs indicating misfolding, and to bind CD81. To delineate the amino acids (aa) regions responsible for proper folding and CD81 binding, several Gla-H77c E2660 chimeric glycoproteins were constructed. Analyses of the chimeras show that (1) the C-terminal region of Gla E2660 (aa 524-660) induces formation of disulfide-linked misfolded aggregates which fail to bind CD81, (2) the hypervariable region 1 of Gla E2660 has no effect on H77c E2660-CD81 interaction, and (3) the aa region 406-524 of Gla E2660 modulates H77c E2660-CD81 interaction without affecting global conformation. Comparison of Gla and H77c E2660 aa sequence with the corresponding sequences of a number of genotype 1a and of cross-genotype

HCV strains has identified variant amino acids within the Gla sequence, which may be responsible for misfolding and failure to bind CD81. Site-directed mutagenesis of the critical aa residues that have been tentatively identified above is in progress, which should enhance our understanding of the mechanisms involved in these processes.

1700 Inhibition of cAMP-dependent protein kinase by the hepatitis C virus NS3 protein

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The hepatitis C virus non-structural protein NS3 is a 70kDa polypeptide that has both protease and NTPase/helicase activities that function during viral replication. NS3 associates with NS4A, a 56 amino acid polypeptide that stimulates protease activity. A peptide corresponding to an arginine rich region of NS3 has been shown to inhibit the activity of cAMP-dependent protein kinase (PKA) *in vitro* and

length, enzymatically active NS3/4A has been expressed with an N-terminal hexahistidine tag using the baculovirus system. The purified protein inhibited the activity of *E.coli* expressed PKA catalytic subunit *in vitro*, measured using a Kemptide kinase assay. Mutations in either the arginine rich motif or an Asp-Glu motif in the NTPase active site abolished the ability of NS3 to inhibit PKA phosphorylation. Secondly in order to investigate the physiological consequences of NS3 mediated PKA inhibition we have used inducible expression vectors to generate stable hepatocyte cell lines expressing NS3. Preliminary data indicate that NS3 expression inhibits the PKA mediated phosphorylation of the transcription factor CREB. Recent results will be presented.

1715 The role of histone deacetylases on human cytomegalovirus infection

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Initiation of infection of the ubiquitous human herpesvirus cytomegalovirus (HCMV) is influenced by the state of cellular differentiation. The non-permissiveness of N-teratocarcinoma (T2) cells for HCMV is believed to result, in part, from the binding of the cellular transcription factor YY1, to the major immediate early promoter/regulator (MIEP) of the virus. As histone deacetylases HDac1 and HDac3, have been shown to bind YY1, we analysed whether these deacetylases mediated YY1-regulated repression of the MIEP. In T2 cells rendered permissive by retinoic acid (RA) treatment, both HDac1 and HDac3 act synergistically with YY1 to repress the MIEP. Consistent with these deacetylases being co-factors of YY1-mediated repression, the levels of both HDac1 and HDac3 decreased upon differentiation of T2 cells with RA. Additionally, permissive RA-treated T2 cells transiently transfected with HDac1 & HDac3 show a reduced level of HCMV infection, compared to cells transfected with a non-functional form of HDac3. Consistent with this, normally non-permissive T2 cells can be infected by HCMV, following treatment with a deacetylase inhibitor.

THURSDAY 13 APRIL 2000

0900 The ins and outs of plant virus movement

S. SANTA-CRUZ
SCRI, Invergowrie

In many respects plant viruses are very similar to their animal counterparts showing similar genome organisations and using similar strategies for replication and gene expression. However, when it comes to movement, both local and systemic, plant and animal viruses are very different from one another. Intercellular movement of plant viruses occurs via plasmodesmata, small membrane lined channels between plant cells. However, both viruses and viral nucleic acids are substantially larger than the functional channel presented by plasmodesmata, which must therefore be modified, before viral movement can occur. This process, which can involve either structural or functional alteration of plasmodesmata, is performed by viral movement proteins. The long distance movement of viruses through plants involves entry into the phloem, transport through sieve elements and finally unloading of virus into tissues that are sinks for photoassimilate. In many cases this process is facilitated by the viral movement protein, however, other viral proteins with specific functions in long distance movement may also be involved. The talk will discuss general aspects of plant virus local and long distance movement with particular reference to studies of virus transport using a fluorescently-tagged potato virus X.

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Newly synthesized proteins that are translocated into the ER undergo oxidation, folding and oligomeric assembly before transport to the Golgi complex and beyond. These processes are assisted by a variety of folding factors and molecular chaperones that associate with the proteins starting already during co-translational entry and continuing until the protein has acquired its native, oligomeric structure. These factors improve folding and mediate the so called 'quality control' process that ensures that proteins are correctly folded. We have analyzed the folding of several viral glycoproteins (Influenza HA, VSV G-protein, SFV E1 and p62) in living cells and found that each of them follows distinct folding strategies, and makes use of chaperones in different ways. Those with several N-linked glycans are dependent on calnexin and calreticulin, two lectin-like chaperones, and use ERp57 as their thiol oxidase. E1 of SFV which only has one glycan binds preferentially PDI. Both thiol oxidases were found to form transient mixed disulfide bonds with their substrate proteins. Our results indicated, moreover, that the presence of N-linked glycans close to the N-terminus of a protein directed it into a calnexin/calreticulin/Erp57 dependent folding pathway, whereas lack of such modification led into to BiP and PDI mediated pathway.

1100 Herpes simplex virus: Entry and envelopment

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Herpesvirus particles are composed of an icosahedral nucleocapsid, a surrounding matrix or 'tegument' composed of at least fifteen different polypeptides, and a lipid envelope containing more than ten different virus specific membrane glycoproteins. Following attachment of the virion to the host cell surface, the virus envelope fuses with the plasma membrane, and at later times in infection, an infected cell membrane may fuse with an uninfected neighbour. Both of these fusion processes are mediated by virus-encoded glycoproteins. Four of the HSV1 glycoproteins, gB, gD, gH and gL are essential for virus entry; virions which lack these molecules bind to cells but fail to enter, and the expression of these 4 proteins in the absence of other virion components is necessary and sufficient to induce syncytium formation. The route by which HSV1 acquires its envelope glycoproteins during virus assembly and egress remains a contentious issue. Nucleocapsids assemble in the nucleus and bud initially through the inner nuclear membrane. Subsequent events are less well defined; enveloped particles in the ER may either leave the cell by the secretory pathway, or they may fuse out of the perinuclear space and gain a membrane from a post-ER compartment. The construction and characterisation of recombinant viruses in which the expression of essential glycoproteins is targeted to either the ER or the TGN has allowed us to address this question, and support the view that the virus de-envelopes at the outer nuclear membrane, and acquires its final envelope from membranes of the trans-Golgi network.

1145 Entry and exit of vaccinia virus

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Vaccinia virus is an orthopoxvirus and replicates in cytoplasmic factories. Two infectious forms of virion are produced, called intracellular mature virus (IMV) and extracellular enveloped virus (EEV). These virions are surrounded by different numbers of lipid membranes, have different surface proteins, bind to different cellular receptors, enter cells by different mechanisms, have different sensitivities to neutralization by antibody or complement and play different roles in the virus life cycle. IMV particles

and are avirulent *in vivo*. EEV particles may represent less than 1% of infectivity but are important for the long-range dissemination of virus and for virus virulence.

During morphogenesis the first structure visible by electron microscopy is a crescent that contains lipid and virus protein. This grows into a non-infectious spherical immature virion (IV) that undergoes proteolytic processing to form an electron-dense, brick-shaped IMV. IMV particles either remain in the cell until cell lysis, or are wrapped by a double layer of cellular membrane derived from the trans-Golgi network or early endosomes, to form an intracellular enveloped virus (IEV). IEV particles move to the cell surface where the outer membrane fuses with the plasma membrane. If the resultant virion remains attached to the cell surface, it is called cell associated enveloped virus (CEV), or if it is released it is called EEV. Efficient cell-to-cell spread of virus requires the polymerisation of actin from the cell surface beneath a CEV particle to drive the CEV particle into an adjacent cell. IMV enters new cells by fusion of its membrane at the cell surface in a pH-independent process, whereas EEV enters cells by endocytosis and requires a low pH step for release of the virus core into the cytosol. A review of our current knowledge of vaccinia virus morphogenesis, egress and re-entry will be presented.

1400 ASFV capsid recruitment onto internal membranes

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Most enveloped viruses gain a single membrane envelope by budding into intracellular membrane compartments. A second mechanism of envelopment described recently for pox viruses, herpes viruses and African Swine Fever virus is more complex, and involves the wrapping of virions by membrane cisternae derived from specific compartments of the secretory pathway. Wrapping is mechanistically very different from budding. Wrapping provides two membranes in one step, and leaves the virion free in the cytoplasm. When compared with budding, wrapping reverses the orientation of envelope proteins in the virus such that the domains of envelope proteins that were located in the lumen of the wrapping organelle are confined to the interior of the virion, while cytoplasmic tails are exposed on the virion surface.

We have been studying the envelopment of African Swine Fever (ASF) virus as a means of understanding the cell biology of wrapping. ASF virus is a large DNA virus that shares the striking icosahedral symmetry of iridoviruses and the genomic organization of poxviruses. Virus particles contain at least 30 proteins and are assembled in cytoplasmic foci called viral factories. We have followed the biosynthesis and subcellular distribution of the major capsid protein, p73, of ASF virus. One of the first identifiable steps in virus assembly is the translocation of 50% of the newly-synthesized pool of p73 from the cytoplasm to the cytoplasmic face of the ER. There then follows a lag time of 1 to 2 hours after which the ER-bound p73 is enveloped. Envelopment requires cellular ATP, GTP and an intact ER calcium store. By using sucrose gradients to follow the size of complexes containing p73 we have been able to show that the ER bound, but not the cytoplasmic pool of p73, forms large complexes of 50,000kDa with kinetics that closely follow envelopment. Complexes of the same size were detected in virions secreted from cells.

The results suggest that the capsid forms by progressive assembly of as many as 700 copies of p73 into a protein complex on the cytoplasmic face of the ER. Learning how interactions between viral structural proteins on the membrane lead to the ordered bending of ER cisternae into icosahedral structures presents a major challenge for future work on the assembly of ASF virus.

1445 Rinderpest C protein interacts with components

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Rinderpest virus of the family Paramyxoviridae encodes eight known proteins, six of which have characterised functions and have been shown to be present in the mature virus particle. The two remaining proteins termed C and V, have undefined functions and are believed to be non-structural in that they have not been shown to be components of the mature rinderpest virus particle. The C and V proteins are both translated from mRNAs of the P gene through accessing alternative open reading frames.

The C protein can be seen to co-localise with components of the viral nucleocapsid in infected cells. We have used the yeast two-hybrid system to demonstrate specific interactions of this protein with both the viral RNA-dependant RNA polymerase (L) and the nucleocapsid protein (N), and confirmed these findings by expressing individual proteins in mammalian cells.

OPEN PAPERS

1500 Cleavage of translation initiation factor eIF4G during picornavirus infection does not involve caspases

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Infection of cells by many picornaviruses results in the rapid inhibition of cellular protein synthesis due to cleavage of the translation initiation factor eIF4G. The poliovirus (PV) 2A and the foot-and-mouth disease virus (FMDV) L proteases are each sufficient to mediate this cleavage but the cleavage mechanism may be indirect involving unidentified cellular protease(s). eIF4G is also targeted for cleavage during apoptosis by caspase-3. We have demonstrated that caspase peptide inhibitors do not inhibit the cleavage of eIF4G during PV or FMDV infection. Similarly, in transient expression studies the cleavage of eIF4G induced by PV 2A or FMDV L was unaffected by these inhibitors. Furthermore, the cleavage of eIF4G was observed in PV-infected MCF-7 cells lacking caspase-3. These data, and the fact that induction of apoptosis yields different eIF4G cleavage fragments, indicate that caspases do not have a major role in the cleavage of eIF4G during PV or FMDV infection.

1515 Identification of cellular proteins that interact with HIV-2 genomic RNA

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The untranslated leader region of retroviral genomic RNA is involved in several essential steps in the virus life cycle including RNA dimerisation and encapsidation. While much research has been directed towards studying interactions of viral proteins with the leader region, relatively little is currently known about cellular proteins that interact with viral RNA during the retroviral life cycle. We have used several different approaches to identify cellular proteins that interact with HIV-2 virus RNA. One of the cellular proteins identified by north-western screening of a T-cell cDNA library was IκB. The ability of IκB to interact specifically with HIV-2 RNA was confirmed *in vitro* by RNA-protein binding assays and we also confirmed a functional interaction between HIV-2 RNA and IκB in activation of NFκB using transfection assays. We propose a novel mechanism in which expression of HIV-2 viral RNA in the cytoplasm of an infected cell results in the disruption of the complex between IκB and NFκB, leading to increased translocation of NFκB to the nucleus and activation of transcription.

1600 Mechanisms of RNA encapsidation in human immunodeficiency Virus Type 2

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It has previously been shown that, unlike Human Immunodeficiency Virus Type 1 (HIV-1), the major RNA packaging signal (psi) of HIV-2 is located between the primer binding site and the major splice donor (McCann & Lever, J.Virol 1997). Thus, both viral genomic and spliced RNAs contain psi. The virus must have a mechanism to specifically select its genome for encapsidation. We have recently shown that HIV-2 achieves this via a co-translational mechanism in which only RNA also coding for the viral core polyprotein Gag is selected for encapsidation (packaging *in cis*) (Kaye & Lever, J.Virol 1999).

We performed further deletion mutagenesis to locate psi in HIV-2 more accurately. Deletion of a region from 380-408nt in the leader reduces encapsidation efficiency to 5% of the wild type level. Protein production and processing is normal in all of the deletion mutants, consistent with the mutations solely affecting packaging. Encapsidation efficiency of psi mutants was further reduced when co-expressed with wild type virus, suggesting that the level of available Gag in the cell may be limiting. HIV-2 vectors with an intact psi that do not produce Gag were also shown to compete for packaging with psi mutants which do produce Gag, and so be efficiently encapsidated *in trans*. The demonstration of efficient *trans* packaging of HIV-2 vectors allows development of this Lentivirus as a gene vector.

1615 Involvement of the actin cytoskeleton and lipid raft domains in influenza virus budding

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Influenza virus produces virions of two distinct morphologies: pleomorphic spheres with an average diameter of around 100 nm, and filamentous particles of the same diameter but with lengths of up to several μm. The mechanisms underlying the budding process remain largely uncharacterised, but previous work has shown that production of filamentous virions from polarised epithelial cells can be blocked by the actin-disrupting drug cytochalasin D. In addition, it has been suggested that influenza virions bud from specialised domains of the plasma membrane known as lipid rafts. To further understand the mechanism of virus budding we have studied the effect of jasplakinolide, a drug which inhibits actin depolymerisation, on the production and morphology of filamentous virus using atomic force and confocal microscopy in conjunction with a GFP-M1 construct. Jasplakinolide effectively blocked the production of filamentous virus particles, and instead caused the formation of annular structures on the cell surface which contained HA and M1. Consistent with the hypothesis that the virus buds from lipid raft domains, viral filaments and the drug induced ring structures were resistant to extraction with TX-100 at 4°C. Furthermore, both viral filaments and the annular structures contain DAF, a cellular raft associated protein. We propose that an intact actin cytoskeleton is necessary to maintain the correct organisation of raft domains for filamentous influenza virion formation.

1630 Studies of HSV-1 gene expression in the murine central nervous system

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The HSV-1 LAT-promoter (LAP) can drive long-term reporter-gene expression in the peripheral and central nervous systems. To study the kinetics and ability of the LAP to function in different types of CNS neurones, mice were infected with 2×10^6 pfu of either SC16-L A (which expresses LacZ under the control of LAP) or SC16-C3b (in which LacZ is driven by the CMV-IE promoter). Brainstem and spinal cord sections were examined histochemically for LacZ expression from 5 days to 1 year post-infection (pi). CNS neurones were labelled by the CMV-IE promoter from 5-15 days pi only, whilst the LAP labelled identical sites from 7 to 34 days pi. However, after 34 days the numbers of LAP-labelled cells decreased and long-term labelling was predominantly detected in facial, hypoglossal and spinal motoneurones. These results suggest the efficiency of long-term LAP activity may be influenced by the neuronal cell type in which latency is established.

1645 Neutralization of the extracellular enveloped form of vaccinia virus

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Vaccinia virus produces two infectious forms of virus called intracellular mature virus (IMV) and extracellular enveloped virus (EEV). EEV is important for long range dissemination of the virus. Measurement of neutralizing antibodies against EEV was complicated by the contamination of EEV preparations with IMV and the fragility of the EEV outer membrane. Rather than use purified EEV in which the outermost envelope is damaged, fresh EV is prepared from tissue culture supernatant and the contaminating IMV is neutralized by an IMV specific monoclonal antibody. Here we have used four different rabbit antisera and their purified IgG and show that EEV can be neutralized by antibody from natural infection. Furthermore, in agreement with Galmiche *et al.*, (Virology 254, 71-80, 1999), EEV can be neutralised with antibody specific to B5R, an EEV protein. B5R is a member of the family of complement control proteins that is characterised by the short consensus repeat (SCR) module. Using a variety of mutants in B5R, the neutralization target was located to within the stalk region of the protein between the virus membrane and the SCR domains, but not SCR domains, 2, 3 or 4.

1700 Specific and non-specific protection against mucosal challenge with SIV induced by immunisation with recombinant modified vaccinia virus Ankara (MVA)

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Highly attenuated vaccinia virus strains such as recombinant MVA represent a safe and promising approach to AIDS vaccine development. We have tested the ability of MVA constructs expressing structural and regulatory genes of SIV to elicit protective immunity in macaques against rectal challenge. Following intramuscular immunisation of 4 macaques with constructs expressing *rev*, *tat*, *nef*, *env* and *gag-pol* (MVA-SIV), animals made only transient lymphoproliferative and antibody responses; however all animals made CTL responses. Eight weeks after the final immunisation, vaccinates together with 4 naïve controls and 4 animals that had received wild-type MVA (MVA-WT) were

animal, suggesting a self limiting infection. In contrast virus was isolated from only 2 MVA-SIV vaccinates where despite anamnestic anti-env antibody responses virus loads were not reduced compared to controls. Taken together the results show that MVA can efficiently stimulate CTL and prime antibody responses. Protection was associated with persistence of a nef-specific CTL response prior to virus challenge; however, vaccination with MVA-WT also modified the outcome of virus challenge.

1715 Immunosuppression by morbilliviruses

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A major factor influencing the high mortality and morbidity associated with morbillivirus infections is their ability to induce transient immune suppression in the host. This enables secondary pathogens to flourish. A hallmark of such virus induced immunosuppression is the reduced capability of freshly isolated peripheral lymphocytes to proliferate in response to mitogens and recall antigens. An *in vitro* system, developed by Schlender *et al.* (1996 Proc. Natl. Acad. Sci. USA 93 13194-13199), showed the impaired proliferation response of naïve peripheral blood lymphocytes (PBLs), as well as a lymphoblastoid B cell line (BJAB), to a variety of stimuli, after co-cultivation with MV-infected, UV-irradiated presenter cells. We have carried out experiments, based on this system, which show that all members of the morbillivirus genus can impair proliferation of BJAB cells *in vitro*. Proliferation of freshly isolated bovine and caprine PBLs is also inhibited by inactivated rinderpest virus (RPV) and peste-des-petits ruminants virus (PPRV). We have also shown in experiments using recombinant viruses expressing RPV glycoproteins, that as for MV, both the virus haemagglutinin and fusion proteins are required to induce suppression of proliferation of the responder cells. Initial results indicate that this mechanism may be important *in vivo* as PBLs, obtained from cattle and goats infected with RPV or PPRV respectively, also exhibit this effect on responder cells.

FRIDAY 14 APRIL 2000

0900 Crossing the membrane with the single-stranded DNA filamentous bacteriophage

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The Ff class of filamentous phage are composed of a circular single-stranded DNA encased in a protein tube composed of five capsid proteins. Infection is initiated by the specific binding of one end of the phage particle to the tip of the F conjugative pilus. Withdrawal of the pilus brings the tip of the phage to the surface of the bacterial envelope, where the phage tip interacts with TolA, the coreceptor for infection. TolA is part of the TolQRA inner membrane complex, which normally is involved in maintaining the integrity of the outer membrane. The TolQRA complex facilitates the translocation of the DNA into the cytoplasm while allowing the major capsid proteins to dissolve into the inner membrane.

Assembly also is a membrane associated event requiring the five capsid proteins, three phage specific non-capsid assembly proteins, thioredoxin, ATP, a proton motive force and at least one bacterial protein, thioredoxin. The five capsid proteins are synthesized as integral inner membrane proteins which assemble around the DNA as it is extruded through the bacterial envelope. This occurs at a site where the

infection. The known and inferred interactions in the membrane between the phage and bacterial proteins during the infection and assembly processes will be discussed.

0945 Molecular interactions between the envelope components in the assembly of coronaviruses

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Coronaviruses are enveloped, plus-stranded RNA viruses that occur in various mammalian and avian species including humans. The lipid membrane surrounding the nucleocapsid usually carries three different (types of) proteins: M, S, and E. When the genes encoding these proteins are coexpressed in cells, virus-like particles (VLPs) are produced that are similar to normal virions. Actually, the S protein is not required to generate the particles. We have used the VLP assembly system to analyse the interactions between the viral membrane proteins concentrating particularly on the M and the S protein. As part of these studies we observed that the ectodomain of the S protein can be replaced by that of another coronavirus. We subsequently introduced a similar ectodomain replacement in a coronaviral genome which generated a recombinant virus that had lost its authentic targeting properties and had acquired a new tropism. Finally, we investigated the properties of the E protein. An overview of the combined results will be presented.

1100 How do retroviruses encapsidate their RNA genomes

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Retroviruses are, uniquely amongst viruses, diploid. They encapsidate two copies of their RNA genome into each virus particle. These RNAs are linked at their 5' ends in a structure which involves interactions between palindromic sequences in parallel strands. In HTLV-1 a 14 nt core region acts as a major DLS component whose structure can be disrupted by competitor sense, but not antisense oligonucleotides.

Retroviruses encapsidate full length unspliced RNA which also codes for the gag and pol genes. Encapsidation signals consisting of folded regions of the viral RNA are found at the 5' end of the genome. In HIV-1 the major signal is found 3' of the splice donor in a region unique to the unspliced mRNA. In HIV-2 (and SIV) the major signal appears to be 5' to SD in a region common to all the RNAs. HIV-2 solves the problem of specificity of capture by cotranslational packaging. In HIV-1 the NMR structure of the packaging signal demonstrates a complex bulged metastable stem loop. Biochemical analysis of the protein/RNA capture indicates that Gag binds preferentially to unpaired purine motifs and unwinds the helical regions to assemble along the genome.

1145 Molecular interactions from entry to exit of a complex dsRNA virus

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Members of the Reoviridae family share similar morphological and physiochemical properties. These viruses consist of 10-12 discrete segments of double-stranded (ds) RNA genome that are encapsidated by multi-layered protein components but lacking lipid envelope. Unlike the other members of the family, the orbiviruses are vectored to vertebrate species by arthropods replicating in both hosts. How these viruses are able to enter, replicate and spread from cell to cell in a wide range of hosts has been a major theme of my research.

To understand these processes, we have used a particular orbivirus, Bluetongue virus (BTV), as a model. Expression of each gene product of BTV in isolation has allowed us to assay their individual biochemical and structural features. This has resulted in understanding the functional relationship of "who

have been possible to define. Most interestingly, we have been able to reconstitute the transcription/replication complex that has allowed us to understand the processes required for replication of a dsRNA genome. Further, we have unravelled a novel mechanism of virus release in which both a cellular protein and viral non-structural protein act cooperatively for non-lytic release of these non-enveloped viruses. This may open up an understanding of non-cytopathic egress of viruses in vector cells.

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