PROCEEDINGS OF THE PATHOLOGICAL SOCIETY OF GREAT BRITAIN AND IRELAND

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ABSTRACTS OF MICROBIOLOGICAL INTEREST

SYMPOSIUM: CURRENT ISSUES IN SOLID ORGAN TRANSPLANTATION

Chairman: C. G. Gemmell, M. Farrington

PRINCIPLES OF XENOTRANSPLANTATION

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IMMUNOSUPPRESSING THE RECIPIENT OF A PIG XENOTRANSPLANT

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Given the world-wide shortage of organs for human allotransplantation, the pig is considered a potential candidate as a source of organs for man. However, hyperacute rejection – due to activation of human complement – has prevented the use of pigs as organ donors. The action of endogenous complement in man is usually blocked by cell-surface proteins which are regulators of complement activation (RCA). The aim of this research programme was to produce pigs transgenic for human decay accelerating factor (hDAF) – one of these human RCAs. Transgenic pigs have been produced that expressed hDAF in all the different tissues and organs of the pigs without causing any harmful effect to the animal in terms of well being, growth, sexual maturity and reproduction. Subsequently, organs from these pigs were transplanted both with and without immunosuppression into non-human primates. These studies show: (1) Hearts and kidneys from transgenic pigs are not hyperacutely rejected by Cynomolgus monkeys. In a study of 200 pig to Cynomolgus monkey organ xenografts, 0 of 168 transgenic organs were hyperacutely rejected whereas 21 of 32 non-transgenic organs did undergo hyperacute rejection ($p < 0.0001$). (2) Survival of such xenografts up to 3 months can be achieved by immunosuppressing the recipient with drug regimens similar to those which could be used in man. (3) Pig hearts and pig kidneys are life supporting for primates. (4) Studies are being undertaken to assess the risk of transmission of pig endogenous retrovirus by xenografts. Xenotransplantation from transgenic pig donors may provide a solution to the organ shortage crisis.

PORCINE ENDOGENOUS RETROVIRUSES AND XENOTRANSPLANTATION

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Recent interest in the use of porcine organs for xenotransplantation has highlighted the need to characterise the properties of the endogenous retroviruses of pigs. Analysis of various pig cells has allowed us to identify three classes of infectious endogenous retrovirus (PERV-A, -B, -C). These three classes differ significantly in the sequence of the host range determining regions of env but otherwise are highly homologous. Host range studies reveal that PERV-A and PERV-B infect cells from a number of different species whereas PERV-C appears to be more restricted.

PERV-A and PERV-B were shown to infect human cells in culture. Studies of the proviral content of pigs of different origins show that multiple copies of PERV-A and PERV-B are widely distributed in pigs. They are, therefore, likely to be present in donor animals for transplant procedures. We are currently cloning and characterising a number of endogenous proviral clones. RT-PCR studies indicate that transcription of these proviruses can occur in a range of normal porcine tissues. The issue of whether these proviruses present a potential problem for xenotransplantation was discussed.

THE BACTERIAL RISKS OF XENOTRANSPLANTATION

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The greatest perceived infection risk of xenotransplantation is that from porcine viruses. Bacteria introduced with the graft, however, may cause devastating infection as we have learnt from our experience with human allotransplants. Transgenic pigs have been bred from high-health herds. Piglets are delivered by hysterotomy and reared in a controlled environment. They are free of a defined group of organisms known to cause zoonotic infection. Transplantation, however, bypasses the normal routes for acquiring zoonoses. We have to assess the risks of bacterial

contamination at organ harvest and the effectiveness of strategies to reduce that risk. To determine the optimal antibiotic prophylaxis for a recipient, we need to know more about the normal flora that develops in these Specific Pathogen Free pigs and how organisms are acquired. As xenotransplantation can be planned, it is possible to screen an animal to a level not possible for most human donors. The aim should be to produce a xenotransplant free of pig-derived bacteria. By careful controlled harvest, we could also reduce contamination from staff and the environment.

ASPERGILLUS INFECTIONS IN HEART AND LUNG TRANSPLANT PATIENTS

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Aspergillus spp. are one of the most frequently encountered classes of fungi responsible for disease in cardiopulmonary transplant recipients. The pattern of presentation depends on the type of transplant received (isolated heart vs single/bilateral lung vs heart-lung). In cardiac transplant recipients, aspergillus infection is almost always associated with invasive disease most commonly found in the lung. The presentation is often asymptomatic with a nodular shadow (or shadows) noted on a chest X-ray taken for other reasons. A biopsy of these lesions often shows changes consistent with old pulmonary infarcts which have probably become secondarily infected with aspergillus. Treatment with liposomal amphotericin with or without follow-up itraconazole therapy is almost always effective in eradicating the disease if diagnosed early enough. In lung transplant patients where a bronchial anastomosis is performed (i.e., single or bilateral lung transplantation) aspergillus infection is a major cause of subsequent airway complications. The presence of aspergillus in this population implies airway problems and if left untreated the angio-invasive tendency of aspergillus will lead to mycotic aneurysm formation and rupture of the underlying pulmonary artery. Where no airway problems exist, which is usually the case with heart-lung transplant recipients, aspergillus disease is rare early, although saprophytic colonisation of the respiratory tract is common. The presence of aspergillus in this setting does not in itself indicate a need for therapy unless the patient is subjected to augmented immunosuppression such as may occur at the time of an acute rejection episode. Invasive parenchymal disease in lung transplant recipients is almost always fatal despite early therapy with amphotericin. This usually occurs either (early) when immunosuppression is at its highest levels or (late) when obliterative bronchiolitis is present with resulting airway obstruction. Occasionally, patients with aspergillus colonisation will present with typical findings of allergic bronchopulmonary aspergillosis (tissue or airway eosinophilia, asthma, etc.) or will develop ulcerative tracheobronchial aspergillosis. These two forms are not usually fatal. ABPA usually responds to steroids and reduction of aspergillus load with itraconazole. Ulcerative tracheobronchitis usually responds to amphotericin and/or itraconazole therapy.

In conclusion, aspergillus remains an important cause of morbidity and mortality in cardiopulmonary transplant recipients. The use of liposomal amphotericin B has improved survival in invasive disease in both groups, although invasive disease in lung transplant recipients remains highly fatal. Appropriate use of prophylaxis and prompt diagnosis are the keys to controlling this condition.

Data were presented on the outcome of treatment with liposomal amphotericin B and some of the rarer presentations of aspergillus infection in this patient population.

ANTIVIRAL PROPHYLAXIS IN TRANSPLANTATION

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There are many viruses that cause symptomatic infection in transplant patients. For many of these, the severity of disease depends on the amount of immunosuppression being given. The herpesviruses, cytomegalovirus (CMV), herpes simplex virus (HSV) and varicella-zoster virus (VZV) are the most important viruses causing disease in transplant recipients. Fortunately, all three viruses are amenable to treatment with several antiviral drugs. These drugs can also be used prophylactically to reduce the impact of these infections.

The first antiviral drug to have a beneficial effect against CMV was aciclovir. A study employing aciclovir 800 mg qds for 3 months prophylactically in kidney transplant recipients showed beneficial effect. By contrast, early studies with I.V. ganciclovir (5 mg/kg bd for 4 weeks) were disappointing, with no benefit being demonstrated in reducing the impact of donor-acquired CMV. Some benefit was shown in CMV reactivation/reinfection. More recently oral ganciclovir (1 g mtds for 12 weeks) has been shown to have a significantly beneficial effect in reducing the severity of CMV infections in liver, heart and lung transplant recipients.

Aciclovir prophylaxis is given to those HSV antibody-positive transplant recipients (e.g., heart-lung and bone marrow) who are at increased risk of life-threatening HSV pneumonitis. Antiviral drugs are not given routinely to patients to prevent VZV infection. However, VZV antibody-negative transplant patients in contact with chickenpox or zoster may be given aciclovir prophylactically as well as zoster-immune globulin.

Several antiviral drugs are available to ameliorate life-threatening herpesvirus infections. Other antiviral drugs are currently being evaluated prophylactically for herpesvirus infections and may prove to be of benefit.

EPSTEIN-BARR VIRUS AND LYMPHOPROLIFERATIVE DISEASE

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Epstein-Barr virus (EBV), a B lymphotropic herpesvirus with cell growth transforming ability, is widespread in human populations. Primary infection occurs either in childhood, where it is usually asymptomatic, or in adolescence, where it is often clinically manifest as infectious mononucleosis; thereafter the virus is carried for life as a largely asymptomatic infection. However, in immunocompromised individuals, weakening of the cytotoxic T lymphocyte (CTL) response that normally controls EBV-driven B cell proliferation renders the patient at risk of virus-positive lymphoproliferative disease.

In the setting of transplantation, several factors influence lymphoproliferative disease risk, in particular the degree of immune suppression and the patient's prior history of

exposure to the virus. Current efforts are focussing on (i) the development of virological assays that can predict disease risk in individual patients, and (ii) the feasibility of adoptive CTL transfer as an immunotherapeutic procedure.

HUMAN HEART VALVE BANKING: TROUBLE IN STORE?

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Microbiological regulation of allograft heart valve transplantation has concentrated on the virological risks, and there is wide variation in the performance of bacteriological quality control of harvest and processing. Many valves are resected in mortuaries, up to 48 h after donor death, hence bacterial contamination is likely. Homograft valves are decontaminated in antibiotic mixtures, but there is concern over their efficacy against bacteria with novel resistance mechanisms. Transmission of mycobacteria and *Candida albicans* with valve grafts has been reported; by analogy with published results with other tissue transplants, there are also other bacterial risks faced by recipients of homograft valves.

In Cambridge during the past 3 years we have developed a protocol for decontamination, bacteriological monitoring and interpretation of results. This includes cultures performed both at the time of harvest as a measure of 'raw material' quality, and after processing. Recent theatre-procured valves uncommonly fail our acceptance criteria at harvest (4.5%; pure growths of *Candida* and *Pseudomonas* spp.) whereas 13.6% of mortuary-derived valves fail (frequently mixtures of enterobacteria, pseudomonads and staphylococci). In contrast to other published experience, terminal cultures have been sterile. Our decontamination regimen includes gentamicin, imipenem, vancomycin, nystatin and polymyxin, and we advocate its use together with our monitoring protocol and interpretation guidelines.

INFECTIOUS COMPLICATIONS FOLLOWING SMALL BOWEL TRANSPLANTATION

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Infectious complications occur at a high rate following small bowel transplantation and are a cause of considerable morbidity and mortality. Small bowel transplantation was first undertaken in this hospital in 1992. The infectious complications of patients who have undergone this procedure since that date were reviewed. The mean age of the six patients (4 male, 2 female) was 33 years (range 20–41) and mean follow-up was 699 days (range 8–1610 days). All patients received intravenous vancomycin, ciprofloxacin and metronidazole for 48 h perioperatively. Ciprofloxacin (oral or iv) was given while gut integrity was in question. Antifungal prophylaxis consisted of low-dose iv amphotericin B followed by oral fluconazole and nystatin mouthwashes.

Two patients had no infective episodes (follow-up of 720 and 90 days). There were 19 infective episodes among the remaining four patients: 8 caused by bacteria – bacteraemia 6 (5 catheter-related), pneumonia 1 and abdominal abscess 1; 5 by fungi – catheter-related fungaemia 1, disseminated candidiasis 2, oesophageal candidiasis 1, pneumocystis

pneumonia 1; and 3 by viral infection – CMV gastroenteritis 2 and influenza A 1. No organisms were isolated from three episodes of clinical infection. Two patients died; death was attributable to infection in one patient. We have seen a high rate of catheter-related and *Candida* infections, highlighting the necessity for improving preventative strategies against these specific infections. The low rate of gram-negative infection due to bacterial translocation seen in this series may be explained, in part, by targeted prophylaxis with systemic ciprofloxacin.

IMMUNOLOGY OF TRANSPLANTATION

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Donor specific tolerance remains an elusive goal in transplant immunology. Rejection of solid organ grafts is most commonly due to cellular mechanisms; however, humoral immunity may be the prominent mechanism in some situations. The immunological mechanisms causing chronic allograft rejection remain poorly understood. The role of NK cells in failure of bone marrow grafts has recently been recognised; however, the role of NK cells in solid organ transplantation remains to be elucidated. Xenotransplantation will require intensive B cell suppression, and NK cells may play an important role in delayed xenograft rejection.

To date, no clinically applicable tolerance induction protocols have been reported, despite some encouraging results in animal models. Cyclosporin inhibits many animal models of tolerance induction, making clinical evaluation of such protocols difficult.

Prevention of rejection currently depends on non-specific immunosuppression, with protocols heavily weighted towards T cell immunosuppression. In renal transplantation and many cardiac transplant programmes, triple immunosuppression with cyclosporin, azathioprine and prednisolone remain standard therapy. In high risk patients, anti-thymocyte globulin may be added as induction therapy, and FK506 and mycophenylate mofetil may replace cyclosporin and azathioprine. Quadruple immunosuppression is commonly used in lung and pancreas transplantation.

LESSONS IN VIRAL HEPATITIS GAINED IN THE TRANSPLANT FIELD

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Patients with viral hepatitis B or C comprise a significant proportion of patients undergoing liver transplantation. For both viruses it has become clear there is a massive risk of graft infection; this can be moderated by the use of immunoglobulin. Regrettably, graft infection is associated with a significant incidence of graft loss following an accelerated course indicating that in the presence of immunosuppression both viruses are cytopathic and emphasising that maintenance of active immunity is critical to controlling the viruses both before and after the transplant. More recent studies have indicated immunosuppressive agents may have a dual effect. By suppressing immune responses they enhance viral replication but many have a direct effect on the viruses themselves which may be adverse or, rather surprisingly, protective. Thus the choice of

immunosuppressive agent in a patient undergoing transplantation with viral hepatitis could be critical. The high levels of replication seen subsequent to transplantation also increase the chance that mutations will arise which perhaps provide a clue regarding critical areas within the virus. Almost all the strategies utilised thus far have been associated with escape mutations and the long-term control of both viral hepatitis B and C subsequent to transplantation remains a genuine challenge.

CONSENSUS VIEW OF THE INTERNATIONAL IMMUNOCOMPROMISED HOST SOCIETY ON THE EPIDEMIOLOGY, PREVENTION, DIAGNOSIS AND MANAGEMENT OF INFECTIONS IN FOLIC ORGAN TRANSPLANT PATIENTS

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ORAL PRESENTATIONS

RANDOM PRIMING RT-PCR FOR GENOTYPING HUMAN ROTAVIRUSES

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RNA was extracted by use of guanidinium isothiocyanate/silica from faecal specimens previously determined to be rotavirus-positive by ELISA, passive latex agglutination or electron microscopy. After reverse transcription (RT) in the presence of random primers, the VP7 and VP4 genes of rotavirus were amplified by PCR with primers complementary to the conserved 5' and 3' ends, and genotyping was performed with type-specific primers located in variable regions of both genes.

The RT-PCR was able to detect 10 copies of RNA/ml of 10% faecal suspension and could differentiate G-types G1, G2, G3, G4 and G9 and P-types P1A[8], P1B[4], P3[9] and P2A[6]. Of 121 specimens analysed by this method, only 8% could not be G- or P-genotyped. The untyped samples were tested again performing the RT reaction with G- and P-specific primers, achieving a 5% increase in sensitivity. Comparing G-genotyping against G-serotyping, 92% were genotyped through random priming RT-PCR (97% after retesting negatives with specific-priming RT), whereas only 64% were serotyped with G-serotype specific monoclonal antibodies.

VP4 ANTIBODY IS NOT A MAJOR CORRELATE OF PROTECTION IN GNOTOBIOTIC PIGLETS AGAINST HOMOLOGOUS ROTAVIRUS INFECTION

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The importance of rotavirus specific immune responses for protection was studied *in vivo* with two porcine rotaviruses of identical G type, but different P type and different pathogenicity. Porcine rotavirus (prv) 4S is classified as G3

[P7] type and is non-pathogenic in pigs whereas prv 4F is a G3 [P19] type and is pathogenic. Four 4-day-old gnotobiotic piglets were vaccinated orally with prv 4S and challenged 14 days later with prv 4F: there were two challenge control and two uninfected control piglets. All four vaccinated piglets were protected from infection and disease whereas the challenge control animals replicated the prv 4F and developed diarrhoea. Serum VP7- and VP4-specific neutralising antibodies were measured: it was found that the vaccinated animals had strong G3-specific antibody, but only weak and not significant VP4-specific antibody responses. As vaccinated animals were protected this suggests that VP4-specific antibody responses are not a major correlate of protection. There was strong correlation of protection with rotavirus-specific IgA responses in the gut the protein-specificity of which remains to be elucidated.

AUDIT OF HIV LOAD RESULTS: DETERMINING THE EFFICACY OF DUAL AND TRIPLE COMBINATION ANTI- RETROVIRAL THERAPY

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The measurement of HIV load is used to monitor the effectiveness of specific anti-retroviral treatment. The inability of the drug(s) to reduce virus load by >0.5 logs may indicate the presence of drug resistant mutants. In a recent audit, in PHLS East, of HIV load results, data detailing anti-retroviral compounds and duration of treatment, CD4 cell count and clinical symptoms were collected. In a total of 31 (51.6%) of 60 patients the virus load, as measured in the nucleic acid sequence based assay (Nuclisens), did not fall during treatment. In detail, there was no significant reduction in virus load in 15 (25.0%) of 60 and a rise in 16 (26.6%) patients. Changes in anti-retroviral therapy, instigated as a response to the results of viral load measurement, were monitored in order to identify the most appropriate combination(s) of anti-retroviral drugs capable of reducing virus load to undetectable concentrations (<50 copies of RNA/ml). Point mutation assays which have been developed to detect mutations associated with anti-retroviral drug resistance are being evaluated in order to allow rational decisions to be made as to which drug in combination to discontinue, which drug to maintain and which drug(s) to add.

MOLECULAR EPIDEMIOLOGY OF VANA RESISTANT ENTEROCOCCI ON A HAEMATOLOGY WARD

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Sixty-six glycopeptide-resistant enterococci (GRE) with VanA phenotype from faecal screens of 15 patients on a haematology ward were investigated for variability in structure of their VanA elements. They comprised 18 *Enterococcus faecalis* demonstrating two pulsed-field gel electrophoresis (PFGE) patterns and 48 *E. faecium* demonstrating eight PFGE patterns. Overlapping fragments of their VanA elements were amplified with 10 pairs of PCR primers and scored for presence or absence and size of products compared with the prototype VanA transposon Tn1546. This identified two elements among the *E. faecalis* and two among the *E. faecium* isolates.

Forty-six isolates were from a single patient and were analysed for plasmid carriage, and plasmid DNA was Southern blotted and hybridised with a *vanA* probe. Some isolates had plasmid-borne VanA elements; others showed evidence only of elements on the chromosome. Combining PCR and hybridisation data with PFGE data allowed division of *E. faecalis* and *E. faecium* each into five distinct groups. VanA elements were transferrable by conjugation, and analysis of transconjugants from one isolate suggested carriage of multiple VanA elements.

These data demonstrate heterogeneity of VanA elements among GRE examined, and suggest determination of structure and location of elements may broaden epidemiological information acquired from PFGE.

PREVENTION OF EBV POSITIVE TUMOURS IN SCID MICE WITH AUTOLOGOUS CYTOTOXIC T LYMPHOCYTES (CTL)

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Epstein-Barr virus (EBV) establishes persistent infection in over 90% of individuals and is associated with fatal B cell lymphoproliferative disease (BLPD) in 1–10% of solid organ transplant recipients. Severe combined immunodeficient (SCID) mice serve as a murine model for BLPD since both human EBV positive lymphoblastoid cell lines (LCLs) and peripheral blood mononuclear cells (PBMC) regularly give rise to aggressive tumours in these mice.

The aim of this study was to evaluate the role of autologous CTLs in the prevention of LCL-induced subcutaneous tumours in SCID mice, and to establish the minimum dose of CTLs required to prevent tumour outgrowth. LCLs and EBV-specific CTLs were generated from a panel of ten EBV seropositive donors. The number of LCL cells required to consistently give rise to subcutaneous tumours in SCID mice as 2×10^6 . Duplicate subcutaneous injections of combined CTL and LCL were given at ratios of 2:1 down to 0.03:1. Control mice received either CTL or LCL. All LCLs on their own rapidly gave rise to tumours, whereas CTLs did not. CTLs prevented tumour outgrowth down to the CTL:LCL ratio of 0.25:1. The results suggest that immunoprevention of BLPD in transplant patients may be achieved by infusion of autologous EBV-specific CTLs.

GLYCOPEPTIDE SUSCEPTIBILITY TESTING OF COAGULASE-NEGATIVE STAPHYLOCOCCI

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Coagulase-negative staphylococci (CNS) are an increasingly important cause of bacteraemia, particularly in immunocompromised patients. Teicoplanin resistance in coagulase-negative staphylococci is increasing, notably among *Staphylococcus haemolyticus*. Reports suggest that technical problems in teicoplanin susceptibility testing by disk-diffusion may result in failure to detect resistance. In this study the reliability, under a variety of conditions, of teicoplanin susceptibility tests by diffusion and dilution methods was examined.

198 strains of CNS were tested, covering a range of susceptibility to teicoplanin. MICs were determined by agar dilution on Iso-Sensitest (IS, Oxoid), DST (Oxoid) and Mueller-Hinton (MH, Difco) media with inocula of 10^4 and 10^6 cfu. Disk diffusion tests were performed on the same media as for MICs, with inocula standardised to a 0.5 McFarland standard and to semi-confluent growth of colonies, and with 5 µg and 30 µg teicoplanin disks.

With an inoculum of 10^4 cfu, MICs were generally higher on DST and lower on IS agar. With an inoculum of 10^6 cfu, MICs were higher on all media, and differences among media were reduced. MICs correlated poorly with zone diameters. 11–90% of susceptible strains were falsely reported resistant, depending on the combination of test conditions, when zone diameter breakpoints were selected by error minimisation to give <1% resistant strains. The lowest error rates were on IS agar with a semi-confluent inoculum, 5 µg disks, and a zone diameter breakpoint of <12 mm for resistant strains.

In view of the poor reliability of disk diffusion tests, MIC or breakpoint methods might be more appropriate, although the clinical significance of the different degrees of reduced susceptibility of CNS to teicoplanin remains to be established.

ANALYSIS OF THE FLAA GENE OF *HELICOBACTER PYLORI* AND RELATIONSHIP TO CLINICAL DISEASE

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Helicobacter pylori is an important human pathogen infecting about half the world's population. Carriers are at increased risk of developing peptic ulcer disease, gastric adenocarcinoma and gastric lymphoma. The polar sheathed flagella of *H. pylori* are a major pathogenicity factor, required to penetrate the viscous mucus covering the gastric epithelium, and to establish colonisation.

H. pylori strains have been shown to be genetically diverse, and individuals colonised with strains carrying particular alleles of certain genetic loci are at higher risk of developing gastroduodenal disease. *FlaA* is one of the genes encoding structural flagellin antigens. In this study, PCR-RFLP analysis was used to investigate the diversity of *flaA* of *H. pylori* isolates from patients in four different clinical groups (non-ulcer dyspepsia with gastritis; gastric ulcer; duodenal ulcer; and histologically normal mucosa), to

determine if there was a relationship between particular alleles and clinical symptoms.

FlaA was present in all strains. Considerable allelic variation was found within the *flaA* gene of the strains analysed, and no association with individual gastroduodenal pathology was apparent. Investigation continues into the relationship of *flaA* allelic variation to other markers of pathogenicity.

THE PRE-MILLENNIUM BUG?

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Computer networks have become the backbone of hospital information and communication systems in most developed countries. They are adapted to perform various tasks, including electronic patient record handling (laboratory results, radiology, pharmacy, accounting) delivered with improved speed and efficiency in a more legible and

accessible format, with improved speed and efficiency. Tracking patients and their laboratory results is also much easier.

The increasing prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in UK hospitals has resulted in increased patient morbidity, mortality and cost, MRSA can spread from one patient to another via the hands of health care workers. Hands can be contaminated from environmental sources, and these may include computer keyboards. This has obvious infection control implications, as keyboards are often ignored and difficult to clean.

Following the isolation of MRSA from an infected nail bed of one health care worker we sampled computer keyboards in wards and laboratories of two hospitals. The subsequent recovery of MRSA from this source implies the need for appropriate infection control measures, such as the use of commercially available disinfectant-compatible permanent keyboard covers.

POSTER PRESENTATIONS

DETECTION OF HUMAN CYTOKINE MRNA IN EBV POSITIVE TUMOURS IN SEVERE COMBINED IMMUNODEFICIENT (SCID) MICE

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EBV positive B cell lymphoproliferative disease (BLPD) tumours in solid organ transplant recipients are often infiltrated with CD4+ T lymphocytes and preferentially develop in cytokine rich areas such as the transplanted organ or the transplant scar. This suggests that BLPD tumour initiation and/or growth requires factors produced by surrounding stromal cells. For therapeutic purposes, it is important to know whether these factors are produced by the tumour cells in an autocrine fashion or by particular types of stromal cells.

The aim of this study was to investigate the cytokine profile in BLPD tumours by in-situ hybridisation and to establish the cell type producing them. Human peripheral blood mononuclear cell (PBMC) induced tumours in SCID mice serve as a murine model for BLPD and were investigated for the production of IL-2, 4, 6, 10 and INF γ , using digoxigenin labelled oligonucleotide probes. Phytohaemagglutinin (PHA) stimulated PBMC were used as positive controls. All tumours tested were positive for cytokine mRNA, although the pattern of expression and number of positive cells varied between different tumours.

BIOCHEMICAL SPECIATION OF THE GENUS *KLEBSIELLA*

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Recent changes in the taxonomy of the genus *Klebsiella* are not fully covered in the databases of commercial identification kits. There are also no reports that validate an identification scheme to differentiate all seven species

and subspecies of the genus. We used a panel of 273 strains representing these species as well as *Enterobacter aerogenes* and *Pantoea agglomerans*, all of which had been reliably identified by Kiel University, (Germany), Statens Serum Institut (Denmark) or CPHL (UK) together with the nine type strains to validate a set of biochemical tests. The results were used to construct an identification scheme for this genus.

A total of 302 clinical isolates received as *Klebsiella* by us for epidemiological typing was tested with the scheme to determine their identity. 263 isolates (87%) were identified with the following frequencies: 91% *K. pneumoniae* ssp *pneumoniae*, 1% *K. pneumoniae* ssp *ozaenae*, 5% *K. oxytoca*, 1% *K. planticola* and 2% *Enterobacter aerogenes*. 39 isolates did not appear to represent any of the nine species in the scheme.

Other studies have reported frequencies of 59% and 54% for *K. pneumoniae* ssp *pneumoniae*, 23% and 33% for *K. oxytoca*, 13% and 9% for *K. planticola* and 0.2% and 3% for *K. terrigena*. It is possible that the differences seen in this study result from the fact that isolates sent for epidemiological typing represent a population biased towards those that are antibiotic resistant and/or part of outbreak investigations.

GLYCOSIDASE PRODUCTION BY *ENTEROCOCCUS FAECALIS* ENABLES GROWTH ON A MODEL, HIGH-MANNOSE GLYCOPROTEIN

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Enterococcus faecalis is an opportunistic pathogen that causes a variety of nosocomial infections including bacteraemia, infective endocarditis, urinary tract and wound infections. The mechanisms employed by *E. faecalis* to proliferate *in vivo* are fundamental to its ability to cause

disease. In preliminary studies to investigate the ability of *E. faecalis* to degrade glycoproteins and utilise the released carbohydrates for growth, we have used Ribonuclease B as a model high-mannose type glycoprotein. Ribonuclease B is a 15.5-kDa glycoprotein with a single N-glycosylation site and five high-mannose type glycoforms. The glycans consist of the core pentasaccharide (Man₃-GlcNAc₂) with an additional 2–6 mannose residues (Man₅-Man₉). Man₅ and Man₆ are the predominant glycoforms.

E. faecalis grew on Ribonuclease B with an increase in A₆₂₀ of 0.15. MALDI-TOF mass spectrometry of the glycoprotein after growth showed that the bacterium had degraded all glycoforms down to the asparagine-linked N-acetylglucosamine. These data were supported by SDS-PAGE, which illustrated the deglycosylation of the protein. HPAEC analysis revealed the presence of free glycans in the culture supernate, indicating the presence of endoglycosidase activity.

The ability of *E. faecalis* to degrade and utilise high-mannose type glycans probably facilitates the growth of this bacterium on a variety of host glycoproteins *in vivo*.

DEVELOPMENT OF PROTOCOLS FOR DECONTAMINATION OF ACHILLES AND PATELLA TENDONS WITHOUT THE USE OF GAMMA IRRADIATION AND ETHYLENE OXIDE

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All tendons are terminally sterilised with either gamma irradiation or ethylene oxide if found contaminated after processing. In both procedures, mechanical or chemical changes can occur in the tissues and these have been implicated in some implantation failures. We investigated the alternative of ethanol as a sterilising agent during processing to attempt to eliminate the need for terminal sterilisation.

Tendon pieces without muscle tissue (12 cm² area) were divided into two groups. Group 1 were inoculated with 0.1 ml of a panel containing 2×10^4 cfu of *Pseudomonas aeruginosa*, *Escherichia coli* and *Enterococcus faecalis* and Group 2 were spiked with a second panel of 2×10^4 cfu of *Staphylococcus saprophyticus*, methicillin resistant *S. aureus* and *Micrococcus luteus*.

Some of group 1 (1a) were pre-washed in Triton 0.2% for 15 min and shaken in ethanol 70% for 15 min, and others (1b) were pre-washed and shaken in ethanol 70% for

65 min. Similarly, group 2 samples were shaken in ethanol 70% for 65 min without a pre-wash (2a) or were pre-washed before treatment with ethanol 70% (2b). The tissues were then incubated in nutrient broth overnight and subcultured on selective media.

As many as 38–63% of group 1a tissues were found to be contaminated following incubation whereas only 0–4% of group 1b grew members of the panel. Up to 25% of group 2a tissues remained contaminated but all of group 2b tissues were sterile.

We conclude that tendons must be pre-washed with a mild detergent to release bacteria from the surface of the tissue and to reduce clumping of staphylococcal species. A combination of a pre-wash and exposure for 65 min to 70% ethanol will ensure bacterial killing and eliminate the need for terminal sterilisation.

AN IN-VITRO ASSESSMENT OF THE EFFECTS OF CONSTANT AND EXPONENTIALLY DECREASING CONCENTRATIONS OF QUINUPRISTIN/DALFOPRISTIN AND FIVE COMPARATOR ANTIBIOTICS AGAINST BACTERIAL BIOFILMS

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The effects of quinupristin/dalfopristin and five other comparator antibiotics (ciprofloxacin, vancomycin, teicoplanin, flucloxacillin and erythromycin) against biofilms of eight bacterial strains, five staphylococci and three enterococci, were assessed for bactericidal activity. The bacteria were exposed to the antibiotics at a constant concentration and also at an exponentially decreasing concentration using a pharmacokinetic model, the rate of reduction being matched to the half life of the antibiotic. The drug concentrations used were the MIC and MBC of each strain, previously determined in batch culture.

No significant differences in bactericidal activity were seen with the two methods of exposure, constant and decreasing, the MICs had little if any effect on the biofilm cells, the MBCs had a greater effect with the number of viable bacteria being reduced by up to 2.5 log₁₀. Quinupristin/dalfopristin generally demonstrated a greater bactericidal effect against the enterococci than the staphylococci. The general conclusion of this study was that the bactericidal effect of the antibiotics tested does not differ significantly whether administered at a constant or an exponentially decreasing concentration.