OPEN POSTERS
Adhesion molecules

OP1
The effect of anti-integrin antibodies on the local immune response of mice to *Trichuris muris*
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*Trichuris muris* is a gastrointestinal nematode, which infects the large intestine of mice. Ability to expel varies with mouse strain and depends on the type of immune response elicited. A Th2 response enables expulsion, avoiding a chronic infection. Immune defence against pathogens entering the gut is accomplished by lymphocytes in the gut-associated lymphoid tissue (GALT), and trafficking of lymphocytes to the GALT is known to be dependent on the interaction of the α4β7 integrin with its ligand, mucosal addressin cell adhesion molecule-1 (MAdCAM-1). We have investigated the interactions of α4β7 with MAdCAM-1 along with other potential integrin/addressin interactions, e.g. L-selectin with MAdCAM-1 and α4β1 with vascular cell adhesion molecule-1 (VCAM-1) in C57BL/6 mice, which are resistant to infection. We have shown differences in worm burden and mast cell recruitment to the large intestine after treatment with various combinations of anti-integrin antibodies. We have also investigated potential differences between treated groups in CD4+ recruitment to, or location within the large intestine by immunohistochemistry.

OP2
Effect of ICAM-1 polymorphism on cell surface expression and efficiency of leukocyte adhesion to transfected Cos7 cells, *in vitro*
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Two single base pair polymorphisms have been described for the adhesion molecule ICAM-1, located in exons 4 and 6, modifying codons 241 (G/R; Mac-1 binding domain) and 469 (E/K; aggregation domain). In this *in vitro* study we have made cDNA constructs of each ICAM-1 polymorphic variant by site directed mutagenesis, and transfected them into Cos 7 cells together with a β-galactosidase reporter gene. Using a panel of MAbs (6.5B5, 8.4A6, 7.5C2, RR-1/1-1, MEM-111, MEM-112) directed against different epitopes of ICAM-1 extracellular domain, differences in cell surface expression of each of the four possible variants, G241/E469, G241/K469, R241/E469 and R241/K469 (not present in the UK population), were examined. Increased expression of ICAM-1 of GE genotype was detected with all 6 MAbs (GE P > 0.05 compared to expression of GK or RE). The adhesion of fluorescently labelled PBMC to ICAM-1-transfected Cos 7 was investigated. Adhesion was greatest to Cos7 transfected with ICAM-1 of GE genotype (GE: 48% increase in adhesion above Cos 7 transfected with vector alone; Gk: 17%, RE: 19%, RK: 33%). These data are in agreement with our previous findings in HUVEC.

Allergy

OP3
Influence of epitope density on rat basophilic leukaemia (RBL) cell degranulation
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Specific IgE antibody production is a critical event in immediate type hypersensitivity reactions, including asthma and food allergy. We have examined the utility of the RBL cell line for the measurement of murine IgE responses. This cell expresses high affinity IgE receptors that bind IgE and can be stimulated to degranulate by cross-linking with protein allergen, releasing granules that have been labelled with 3H-serotonin. RBL cells were sensitized with mouse monoclonal antidiitrophenyl (DNP) IgE antibody (1–125 ng/mL) and challenged with DNP-albumin conjugates (10–40 ng/mL) with various substitution ratios (SR). Marked RBL cell degranulation (40 to 60% specific release) was induced by conjugates with SRs of between 16 and 32 molecules of DNP per...
molecule of protein, whereas conjugates with lower SRs of 10 or 3 failed to elicit significant serotonin release. In contrast, all conjugates were able to induce mast cell degranulation in vivo in an homologous passive cutaneous anaphylaxis assay. Thus, successful RBL cell degranulation requires relatively high epitope densities, suggesting that this assay is inappropriate for the routine analysis of specific polyclonal IgE antibody responses.

**OP4**

**Modulation of IgG and IgE antibody responses following oral exposure to ovalbumin**

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Intradermal (id) exposure of BALB/c strain mice to allergenic proteins such as peanut lectin and ovalbumin (OVA) results in the production of high titre IgE antibody. We have investigated the influence of oral prior exposure to OVA on the development of specific antibody responses. Groups of mice were pretreated by oral gavage with 25 mg OVA, 0.25 mg OVA, or water alone, on day 0. On days 7 and 14 animals received 2% OVA by id injection into the dorsum of each ear. Serum was collected on day 21 and analysed by ELISA, or by homologous passive cutaneous anaphylaxis assay for the presence of OVA-specific IgG and IgG1, or IgE antibody, respectively. Intradermal administration of OVA stimulated vigorous IgG and IgG1 antibody production and induced IgE antibody titres varying from 1/4, 1/8 and 1/32 (n = 3 experiments). Prior oral exposure to 25 mg OVA was without marked effect on IgG or IgG1 antibody, but inhibited completely IgE antibody production. Pre-exposure to 0.25 mg OVA resulted in somewhat increased IgG and IgG1 antibody titres and did not impact on IgE antibody titres. These data demonstrate dose-related immunomodulatory activity of oral OVA administration on IgE antibody production.

**OP5**

**DC from allergic mice induce specific IgE in absence of AG and evade T-cell-mediated apoptosis**

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We have investigated the role of DC from allergic mice in the production of allergen-specific IgE antibody after passive transfer into naïve syngeneic recipients and the ability of DC from allergic and control mice to undergo apoptosis following interaction with antigen-specific T cells. DC isolated from spleen and Peyer’s patches (PP) of both cow’s milk (CM) allergic and control mice were transferred into naïve syngeneic recipients and antibody responses were subsequently evaluated. In additional studies DC from allergic and control mice were cocultured in presence of T cells and the level of T cell-mediated apoptosis measured by flow cytometry. DC isolated from spleen and PP of allergic mice induced CM-specific IgE antibody response in naïve recipients even in absence of antigen challenge. Also, splenic DC from allergic mice failed to undergo the same level of apoptosis following interaction with antigen-specific T cells compared with DC from CM-immunized, but not allergic, mice. These data demonstrate that DC play an important role in the genesis of IgE and point to an altered DC-T cell interaction that might be relevant in the regulation of immune responses in allergy.

**OP6**

**Regulation of cytokine expression by allergen-activated lymph node cells (LNC)**

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Topical exposure of BALB/c strain mice to different classes of chemical allergen results in selective activation of lymphocyte subpopulations. We have examined whether the divergent cytokine expression patterns are regulated at the level of mRNA or protein production. Auricular LNC were prepared 13 days after the initiation of exposure to the respiratory allergen trimellitic anhydride (TMA), or to the contact allergen dinitrochlorobenzene (DNCB). Type 1 and type 2 cytokine gene and protein expression were analysed by ribonuclease protection assay and Luminex, respectively. DNCB-activated LNC secreted relatively high levels of interferon (IFN)-γ protein, whereas the converse type 2 pattern was observed following TMA treatment. LNC from TMA-treated mice displayed a marked type 2 cytokine mRNA profile (high levels of interleukins (IL)-4, -10 and -13). In contrast, mRNA isolated from DNCB-activated LNC expressed only low levels of IFN-γ transcripts. TMA-induced type 2 cytokine expression is regulated primarily at the level of transcription whereas DNCB-induced type 1 cytokine secretion is likely to be either the result of the release of stored cytokine or translation of previously transcribed mRNA.

**OP7**

**Aluminium hydroxide elicits a shift from a Th2 to Th1 response by PBMC from allergic subjects**

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Aluminium hydroxide (alum) is a commonly used adjuvant in the specific immunotherapy of allergic disease. Whilst alum is traditionally associated with murine Th2 sensitization, little is known about its effects on secondary responses in humans. We aimed to investigate the in vitro effects of alum on PBMC from 18 atopc patients sensitive to grass pollen. PBMC were stimulated with Phleum pratense in the presence or absence of alum. After six days cytokine production was analysed. Results show that PBMC cultured with 50 μg/mL of alum and 5 μg/mL of allergen displayed a significant decrease in IL-5 production compared with allergen alone (alum + allergen = 530 pg/mL ± 236, allergen = 1024 pg/mL ± 274; P < 0.001). In addition, alum induced a significant increase in the ratio of IFNγ to IL-5 (alum + allergen = 2.15 ± 1.04, allergen = 0.31 ± 0.19; P < 0.001) thus favouring a Th1 response. This switch was not dependent upon IL-12 or IL-4. Other experiments investigating the effects of alum on antigen presenting cells show that alum induced increased expression of CD86 and HLA on monocytes whilst decreasing the expression of CD80. In summary, alum can promote allergen-driven cytokine responses toward a Th1 phenotype.
OP9
A new model for allergy research – the JLA mouse

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T cell clones derived from allergic individuals can be tolerated in vivo. This anergy induction is associated with peptide presentation in the context of MHC class II expression on T cells. The main question arising from this is whether antigen presentation under these conditions can be used as a therapy to treat allergic symptoms. Many mammals express MHC class II on the surface of T cells, however, murine T cells lack MHC class II expression. To address this question, a murine model – the JLA mouse – has been made which constitutively expresses MHC class II on the T cell under the control of the CD2 promoter. Normal development of the mice has been observed, however, differences exist in the development of thymocyte and splenic cells. At 4 weeks of age both JLA and WT mice have comparable T cell development in the thymus and spleen. By 12 weeks JLA mice have a high percentage of CD4+ CD8− T cells in the thymus which are absent in the WT mice and a higher number of splenic T cells concurrent with a reduction in B cell numbers. As both human and rat T cells can present peptide, the question under investigation is whether the JLA T cells can present peptide to both specific hybridoma cells and isolated transgenic T cells.

OP10
Fluticasone propionate increases suppressive activity of human CD4+ CD25− T cells in allergen-stimulated cultures

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CD4+CD25− and CD4+CD25+ T cells were separated from atopic and nonatopic donors by immunomagnetic beads, then cultured with allergen extracts (100 μg/mL) for 6 days. Proliferation was assessed by incorporation of tritiated thymidine. Fluticasone propionate inhibited proliferation of CD25+ T cells in a dose dependent manner with an IC50 of 10−10 M. Suppression of allergen-induced proliferation of CD4+CD25− T cells by CD4+CD25+ T cells (in a ratio of 2:1) was significantly less when cells were obtained from atopic donors (n = 9) compared to non atopic donors (n = 7) (median 44% vs. 81%, P < 0.05). Addition of fluticasone at a dose of 10−11 M had no effect on this suppression. However, preincubation of CD4+CD25− T cells with fluticasone at a dose of 10−7 M for 24 h significantly increased their suppression of CD4+CD25+ T cells (from 33% to 55% for cells from atopic donors, n = 8, P < 0.01, and from 38% to 81% for nonatopic donors, n = 6, P < 0.05 when mixed at a 1:1 ratio). These data suggest that corticosteroids can increase the suppressive ability of human CD4+CD25− T cells and may have relevance to their activity in treatment of allergic disease.

OP11
Monophosphoryl lipid A can promote Th1 cytokine responses of human PBMC stimulated with allergen

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Monophosphoryl lipid A (MPL) is derived from the lypopolysaccharide (LPS) of Salmonella minnesota R 595 and has recently been used as an adjuvant in successful grass pollen immunotherapy. However, little is known about the influence of MPL on cellular responses to allergens. We aimed to study the in vitro effects of MPL on PBMC derived from 13 subjects suffering from grass pollen allergy. PBMCs were cultured with Phleum pratense extract (0, 2, 20 μg/mL) and MPL (0, 10 μg/mL) and after 6 days proliferative responses and cytokine profiles were measured. Results show that MPL induced a significant increase in IFNγ production (allergen alone, 645 ± 466 pg/mL (mean ± SE) vs. allergen + MPL, 3232 ± 818 pg/mL; P < 0.001). In addition, there was a significant decrease in IL-5 production (4307 ± 1030 pg/mL vs. 2997 ± 826 pg/mL; P < 0.01). IL-10 production did not change and proliferative responses were unaffected by the presence of MPL. Addition of neutralizing IL-12-antibody resulted in a 95% inhibition in MPL-induced IFNγ production. In summary, the combination of MPL with grass pollen extract resulted in immune deviation of allergen-induced peripheral T cell responses in favour of Th1.
**OP12**

**Secretion of basogranulin, a novel marker for basophil activation, from human basophils in response to IgE-dependent and non-IgE-dependent stimuli**

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Basogranulin is a constituent of basophil secretory granules that has been identified by the basophil-specific monoclonal antibody BB1. We have investigated the release of basogranulin from purified peripheral blood basophils in response to IgE-dependent and non-IgE-dependent stimuli, and compared them with the release of histamine. Basogranulin, as quantified by a dot blot procedure, and histamine (determined by a glass microfibre-based method) were released in response to anti-IgE antibody with bell-shaped concentration response curves. With calcium ionophore, fMLP and C5a, both mediators were secreted in a purely concentration-dependent manner. Wortmannin, a PI3-K inhibitor, suppressed IgE-dependent mediator release. Half maximal basogranulin release in response to anti-IgE was observed by 30 sec, and maximal release (28%) by 15 min. With all stimuli, the kinetics of basogranulin secretion appeared similar to those for histamine, and levels were closely correlated ($P < 0.0001$). The measurement of this unique basophil marker should be valuable in distinguishing basophil activation from that of mast cell activation.

**OP13**

**Detection of IgE-mediated allergen binding to B-cells using biotinylated grass pollen extract**

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Serum from hayfever sufferers containing antigen-specific IgE is able to facilitate antigen presentation to T-cells, and this activity is blocked by serum obtained following subcutaneous immunotherapy to the relevant allergen. We have previously established a novel FACs-based technique, which directly measures the binding of allergen-IgE complexes to the surface of B-cells using a fluorescently labeled anti-IgE antibody. In recent studies, we have extended this protocol and used a biotinylated allergen extract to measure allergen-IgE binding. In the presence of IgE containing sera from atopic subjects $28.5\pm 12.3$ (mean $\pm$ SD) of B-cells bound to allergen which corresponded to $23.7\% \pm 11.3$ of B-cells with IgE on their surface. Presence of specific IgE was required since allergen binding was undetectable in the absence of atopic IgE-containing serum. Moreover, in the presence of sera from 6 immunotherapy-treated patients, binding of allergen was inhibited by 76.5\% $\pm 7.2$ similar to a $77.8\% \pm 14.2$ inhibition of IgE binding to B-cells. This suggests that allergen binds to B-cells as allergen-IgE complexes, which can be inhibited in the presence of sera from immunotherapy-treated patients.

**Antibodies**

**OP14**

**Measurement of IgG and IgG4 antibodies to rat urinary allergen in sensitized and nonsensitized exposed individuals**

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Several individuals who work with laboratory animals develop allergic reactions to proteins secreted in their urine. Asthma, the most serious manifestation of laboratory animal allergy (LAA) occurs in a large percentage of individuals. The precise role of specific IgG in LAA remains to be determined. Some studies have shown the presence of specific IgG to rat urinary proteins (RUP) is related to clinical symptoms and specific IgE, whereas others have observed that it is more related to the degree of exposure to rat proteins. We measured IgG levels to RUP in cases (defined as having a rat urine RAST of $<2\%$ binding) ($n = 80$) and referents (defined as having a rat urine RAST of $\geq 2\%$ binding) ($n = 643$) that were comparably exposed. We found that cases had significantly more specific IgG than referents ($P \leq 0.001$). Specific IgG4 antibodies were significantly increased in cases ($n = 61$) compared to referents ($n = 57$) ($P \leq 0.001$). In conclusion, these findings indicate that both specific IgG and IgG4 antibodies are related to sensitization with rat urinary protein.

**OP15**

**The rhesus macaque immunoglobulin light chain variable genes**

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Immunoglobulins (Ig) comprise two identical heavy chains, joined with two identical light chains, kappa or lambda. Ig genes are encoded by multiple gene segments; variable (V), diversity (D) and joining (J). The availability of multiple gene segments: combinatorial diversity (different VDJ & V1 combinations), and heavy/light chain pairings, contribute significantly to the antibody repertoire. In humans, there are approximately 35 functional or open reading frame variable gene regions for both IgK and IgL, organized into 6 and 11 subgroups, respectively. Non-human primates are increasingly used as animal models in studies that require the assessment of specific antibody changes. The objective of this work was to examine IgKV and IgLV germline variability in the Rhesus Macaque. Subgroup-specific oligonucleotide primers were designed to amplify rearranged light chain sequences from the genomic DNA of an individual Rhesus Macaque. PCR was performed using a proof-reading polymerase and blunt ended PCR products were cloned. Plasmid DNA was sequenced bidirectionally on a MegaBACE capillary system. Comparative analysis of these sequences will be presented.
OP16
The effect of Ramadan month fasting on the humoral immune response

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A wide range of studies regarding the effect of low calorie diet on rodents reveals its deep effect on health, life expectancy and increased potency of immune system. There are also reports which show the effect of low calorie diet on hindering the ageing of immune system. Bearing in mind that one of the major aspects of Ramadan Fasting is avoidance of eating, and the avoidance leads to low calorie intake; therefore, we were encouraged to study its effects on human humoral immunity. The study was carried out on 38 students who had the same diet. They were taken two blood samples: before Ramadan and at the end of the month. The sera obtained was tested for total level of IgA, IgG and IgM and serum components like C3 and C4 by the use of Single Radial Immunodiffusion method. Results showed that there was not a significant difference between serum levels of IgA, IgG, IgM, C3 and C4 before and at the end of Ramadan (P > 0.05). It is concluded that Ramadan fasting has no effect on humoral immunity.

OP17
Selection and characterization of phage antibodies to HERV-K

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Human endogenous retroviruses (HERVs) have been implicated in carcinogenesis and autoimmunity. HERV-K mRNA has been detected in many carcinoma cell lines however, the significance of HERV protein or retroviral-like particle expression is less well understood. A HERV-K peptide was used to isolate scFv antibodies by selection from the Nissim phage antibody library. An scFv, H10, was selected and shown to bind to the peptide by ELISA. Immunocytometry using soluble H10 scFv showed positive staining of the GH teratocarcinoma cell line, which has been previously shown to produce HERV-K retrovirus-like particles. The GH and other carcinoma cell lines were further examined for expression of HERV-K protein by flow cytometry. GH and T47D cell lines showed the highest level of cell surface expression, LoVo, CaCo-2 and COR-L23 cell lines showed a lower level of staining. Western blotting revealed that H10 bound to a large polypeptide of over 200 kDa in cell lysates, and in the case of the GH cell line, a secreted protein of 60 kDa. This methodology will prove useful in the generation of further HERV-K specific scFv providing a panel of reagents to understand better the role of HERVs in disease.

OP18
Quantification of low concentrations of monoclonal anti-D using flow cytometry


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Flow cytometry (FC) and autoanalyser (AA) technology may be used to quantify anti-D levels in plasma (1). We wish to validate FC against AA for quantification of foeto-maternal transport of monoclonal anti-Ds in the human placental perfusion model. Serial dilutions in isoton (1–500 ng/mL) of an IgG1 anti-D monoclonal (Fog-1G1) and a mutated version lacking FcR binding motifs (Fog-1G1Δnab) provided calibration curves for FC anti-D measurement. Perfusion samples were diluted to attain mean channel fluorescence values to fit the linear portion of the curves. Anti-D binding to D positive RBC was detected using FITC-labelled antihuman IgG. Native and mutated anti-Ds produced almost identical best-fit curves (r² > 0.99) irrespective of storage time. On storage, the shape of the calibration curves changed from hyperbolic to sigmoid. Hyperbolic curves gave excellent sensitivity down to 1 ng/mL while the sensitivity of the sigmoid curves deteriorated at antibody concentrations <10 ng/mL. FC demonstrated a greater sensitivity than AA at concentrations of monoclonal anti-Ds <50 ng/mL. Despite alteration of the IgG Fc end, Fab binding activity of the Fog1G1Δnab antibody was the same as the native form.


Asthma

OP19
Airway epithelial protease activated receptors regulate recovery from insult

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Airway epithelial damage is a characteristic of asthma. The resulting increased penetration of environmental agents may potentiate damage and prolong inflammatory responses. In this study we have investigated the influence of protease activated receptors (PARs) on epithelial recovery following insult. 16HBE 14o- cells were subjected to tight junction disruption by treatment with Ca²⁺-free medium or mechanical scratching and the influence of PAR activation on recovery determined. Trans-epithelial resistance (TER) was used as an index of tight junction recovery after Ca²⁺ restoration and an image analysis technique was used to quantify scratch recovery. PAR1 stimulation modestly enhanced TER recovery but had no effect on scratch healing. PAR2 stimulation inhibited TER recovery and the rate of healing of scratches. PAR4 activation dramatically enhanced TER recovery after 24 h but exerted no influence on scratch repair. Activation of PAR2 deleteriously affects the capacity of epithelial cells to re-form a tight barrier following injury, indicating a possible role for epithelial PAR2 receptors and the endogenous activator, mast cell tryptase, in the pathogenesis of asthma.
OP20
Mast cell tryptase stimulates the generation of inflammatory cytokines by human lung myofibroblasts and fibroblasts: role of protease activated receptor 2 (PAR-2)

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Bronchial asthma is associated with an increased degree of mast cell activation and myofibroblast hyperplasia in the airways. We have investigated the pro-inflammatory actions of the major mast cell product, tryptase on lung fibroblasts and myofibroblasts. The myofibroblast phenotype was induced in primary cultures of fibroblasts and in the MRC-5 cell line by treatment with TGF-β. Tryptase induced the up-regulation of mRNA expression for IL-6, IL-8 and GM-CSF from both fibroblast and myofibroblast cultures, and the release of IL-6 and IL-8 was confirmed by ELISA. The actions of tryptase were inhibited by addition of protease inhibitors, indicating dependence on an intact catalytic site. Immunocytochemistry with antibody P2A, indicated expression of PAR-2 on cells. Peptide agonists of PAR-2 were able to stimulate similar patterns of cytokine generation and release to those for tryptase, suggesting a role for this receptor in mediating the actions of tryptase. Tryptase may be a stimulus for the release of inflammatory cytokines from fibroblasts and myofibroblasts in asthma, possibly acting through PAR-2.

Autoimmunity

OP21
Bioinformatic analysis of endogenous viral agents implicated in multiple sclerosis to determine potential antigenic determinants

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Nerve damage in Multiple Sclerosis (MS) may be a consequence of abnormal immune responses to auto-antigens, such as myelin basic protein (MBP) and certain viruses have also been implicated with this disease. In particular, human endogenous retroviruses (HERVs), have been suggested, although detailed immune responses to these ‘fossil viruses’ is unknown. Hence it is important to ask whether these HERVs possess antigenic regions of potential immunoreactivity that could be predicted. Employing bioinformatics (computational analysis) for hydrophilicity, accessibility and multiple alignments, to determine potential antigenic sites of HERVs implicated in MS and regions of similarity to host proteins and MS related autoantibodies, it was possible to create a list of what appeared to be the most antigenic B cell epitopes. Some homologies between the HERVs sequences and the autoantibodies sequences were found. Interestingly, some proteins connected to the nervous system had similarity to HERVs sequences pinpointed. This work will enable future studies using experimental approaches to investigate a role of HERVs in the autoimmunity and pathogenesis of MS.

OP22
Immune reactivity to heat shock proteins in Type1 diabetes mellitus

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T1 diabetes mellitus (T1D) is considered to be an immune mediated disease. The possible targets are heat shock proteins (hsp60) and peptides (p277). Higher hsp60 antibody level was observed in T1D patients and in case of 83 T1D children elevated hsp60 epitop (LAK peptide) antibody level was found. According to a current hypothesis T cells play role in the development of T1D. A bias toward Th1 immune response was observed in T1D patients where the level of Th1 cytokines was elevated and the level of Th2 was decreased. ELISPOT analysis was used to examine T cell responses to differentiate Th1 cytokines from Th2 cytokines. Seven healthy and 7 control people were investigated. For T cell stimulation we used positive controls (Pl. Tetanus toxoid), LAK peptide and p277. The detected cytokines were IFNγ and IL-13. Furthermore we examined if there is any shift toward Th1 or Th2 response (Th1/Th2). In the case of p277 we found significantly increased Th1 response in patients. There was no significant difference in Th2 response between controls and patients. A significant shift towards Th1 response in T1D for p277 was observed. In the case of positive controls and LAK peptide there was no significant difference in the Th1 and Th2 cell response.

OP23
Autoantibodies to hIAMP2 and hTHN46 in crescentic glomerulonephritis

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Previously, we identified lysosomal associated membrane protein2 (hIAMP2) as novel target for autoantibodies in patients with ANCA associated vasculitis and crescentic glomerulonephritis. We have now expressed h-IAMP2 in E. coli and mammalian cells and used the resultant fusion proteins to develop robust ELISAs. These were used to analyse the clinical significance of anti-hIAMP2 antibodies in two large cohorts of patients. Antibodies to hIAMP2 were detected in 33 of 37 untreated patients (89%) and in 30 of 34 patients with relapses (88%) in the first cohort, and their presence reflected disease activity better than antibodies to Proteinase3 (PR3) or myeloperoxidase (MPO). The autoantibodies recognized epitopes on the protein backbone of hIAMP2 rather than glycoepitopes. The results were confirmed in the second cohort of 75 patients. We extended the study to determine the frequency of autoantibodies to a related glycoprotein, transGolgi network protein46 (hTGN46), which we found to be less prevalent. We conclude that anti-hIAMP2 antibodies are common in ANCA associated disease, that they show a better correlation with disease activity than antibodies to PR3 or MPO and we currently determine their pathogenetic role.

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OP24
Immunotherapy of collagen induced arthritis with the stress protein bip
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Previously we have described the stress protein BiP as a putative autogin in RA and that the administration of BiP intravenously prior to induction of CIA resulted in amelioration of disease. Studies presented here focus on the therapy of CIA and investigate a less invasive route of BiP administration: subcutaneous. Earlier experiments have shown that T cells from DBA-1 mice immunized with BiP in saline produce considerably raised levels of Th-2 cytokines, IL-4, IL-5 and IL-10, as compared to saline immunized controls upon in vitro stimulation with BiP. The subsequent disease studies showed that administering BiP systemically and subcutaneously could significantly reduce the development of CIA in regard to incidence and severity when administered at the onset of disease. At the termination of the study splenocytes and draining lymph node cells were removed from all groups of mice and T cell cytokine secretion was assessed. T cells removed from mice that had been treated with BiP via both routes, were shown to secrete IL-4 in response to in vitro BiP stimulation, suggesting that treatment of CIA with BiP is mediated at least in part by an anti-inflammatory Th2-type response.

OP25
Lymphocyte depletion in multiple sclerosis induces a relative increase in regulatory T cells
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Campath-1H, a monoclonal antibody that depletes T cells, profoundly reduces central nervous system (CNS) inflammation in patients with multiple sclerosis (MS). However, one-third of patients develop autoimmune hyperthyroidism (Graves’ disease) at 12–18 months after treatment. Three months after Campath-1H, the percentage of CD4+ cells expressing CD25hi (‘regulatory T cells’) increases (14.8% vs. 3.4% at baseline, P < 0.0005). This effect is lost at 12 months, but is reproduced with a second treatment. Interferon-beta does not affect this CD4+ T cell population. We hypothesize that the initial relative increase in CD4+ CD25hi cells contributes to the control of antimyelin autoreactivity. However, as the influence of regulatory T cells wanes at 12-months, autoreactive T cells may expand and become activated, resulting in the return of MS disease activity, or the emergence of Graves’ disease to which patients with MS are genetically susceptible.

OP26
MRL/Mp CD4+ CD25+ T cells resist suppression by CD25+ Tregs in vitro: a defect of effector T cells in lupus?
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Little is currently known about the interaction of regulatory T cells (Tregs) with effector cells in lupus. The phenotype and function of peripheral CD4+ CD25+ and CD25+ T cells from the MRL/Mp mouse – a polygenic model of lupus – were therefore examined, prior to disease onset. Comparing CD25+ or CD25- T cells from MRL/Mp mice with those of a haplotype-matched control strain, CBA/Ca, no differences were observed in expression of CD44, CD45RB, CD62L or CD152. The median proportion of CD25+ Tregs required to elicite 50% suppression of proliferation of CD25+ T cells activated by anti-CD3/CD28 beads for 72 h (1,50) was lower in CBA/Ca (1.1%; n = 3) and BALB/c (2.5%; n = 4) than in MRL/Mp (11.6%; n = 3) cocultures. Cross-strain cocultures suggested that the CD25+ T cells were responsible for the higher MRL/Mp I,50s: median I,50 for MRL/Mp CD25+-CBA/CA CD25+ was 1.4% (n = 2), while that for CBA/CA CD25+-MRL/Mp CD25- was 11.2% (n = 2). The MRL/Mp CD25- T cells were not hyperproliferative, yielding similar counts to CBA/Ca cells at 48 and 72 h. These data support an abnormality of peripheral tolerance in MRL/Mp mice, referable to the regulated, rather than the regulatory, T cells.

OP27A
Immunodominant B and T cell epitope in experimental autoimmune glomerulonephritis
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Experimental autoimmune glomerulonephritis (EAG), a model of Goodpasture’s disease, can be induced in WKY rats by immunization with rat alpha 3 chain of type IV collagen (α3[IV]NC1). In patients, the major B cell epitope is located at the N-terminus of α3[IV]NC1, but the T cell epitope has not been defined. To investigate whether autoimmune responses in EAG are directed towards peptides in the same region of α3[IV]NC1, we immunized rats with α3[IV]NC1 (positive control) and five 15-mer overlapping synthetic peptides from the N-terminus of α3[IV]NC1. Positive controls produced an antibody response to recombinant α3[IV]NC1 and peptide 2. Splenic T cells from these animals proliferated in response to α3[IV]NC1 and peptide 2. No antibody or T cell responses were observed to other peptides. Rats immunized with...
peptide 2 developed circulating and deposited antibodies and crescentic glomerulonephritis by week 6. Antibodies from these animals recognized α3(IV)NC1 and peptide 2, while their T cells proliferated in response to peptide 2. Animals immunized with other peptides developed no immune response to α3(IV)NC1 and no disease. These results demonstrate that a 15-mer peptide from the N-terminus of α3(IV)NC1 is recognized by B and T cells from rats immunized with α3(IV)NC1, and that the same peptide can induce EAG.

B Cells

OP28
Vav proteins are required for ERK but not PI3-K activation following IgM cross-linking but are required for PI3-K activation in response to CD19 signalling
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B cell responses are initiated upon cross-linking of membrane immunoglobulin (IgM) by antigen. CD19 is a coreceptor that amplifies and regulates responses to IgM cross-linking. We show Vav1 and Vav2 proteins regulate Ca\(^{2+}\) flux and ERK activation in a PI3K-independent manner when IgM is cross-linked through IgM. By contrast, upon CD19 engagement both ERK and PI3K activation required Vav1 and Vav2 proteins. Moreover, inhibition of PI3K enzymatic activity or deletion of Vav1 and Vav2 proteins has a much more profound inhibitory effect on Ca\(^{2+}\) fluxes when the IgM is coligated with CD19 compared with ligation of IgM alone. Our results show the p110α catalytic subunit and the p85α regulatory subunit have an important contribution to the total PI3K activity elicited by IgM or IgM/CD19 coligation. However, IgM stimulated ERK activation is much less dependent upon p110α and p85α than is IgM/CD19 stimulated ERK activation. Our results indicate that other PI3Ks, possibly p110ζ, contribute to PI3K activity in B cells. In addition, we show that CD19 not only enhances B cell responses but also modifies them in a qualitative manner.

OP29
Antibody subclass switching in the context of malaria
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The study explores antibody class switching, a critical factor in the induction of immunity to malaria, by characterizing the formation of IgG antibodies specific to P. falciparum Merozoite Surface Protein 2 (MSP-2). Immuno-epidemiological studies have demonstrated that MSP-2 elicits a highly polarized IgG subclass profile where IgG3 antibodies predominate in malaria immune adults. This is in contrast to other malaria antigens (e.g. MSP-1) that elicit primarily IgG1 or mixed IgG1 or IgG3 response. The switch to MSP-2 specific IgG3 production has been associated with a decreased risk of clinical malaria in children. We utilized a mouse immunization model with recombinant MSP-2 in which the antibody response is polarized to IgG2b to identify possible antigenic structures which selectively drive IgG subclass switching. We also characterized T cell responses and cytokine production in vitro to the different MSP-2 antigens in an attempt to identify specific subclass switch factors. The phenomenon of the polarized IgG subclass to MSP-2 offers an interesting model in which to explore the factors regulating the mechanisms of antigen-specific antibody subclass switching.

OP30
The IgD-binding domain of the Moraxella IgD-binding protein MID (MID962-1200) activates human B cells and induces class switch independently of T cells
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MID, a 200-kDa outer membrane protein that is found in most Moraxella catarrhalis strains, displays a specific affinity for human IgD. In addition, MID has adhesive capacity and consequently binds epithelial cells. The adhesive part of MID is localized within the 150-amino-acid fragment MID764-913, whereas the smallest fragment with preserved IgD-binding comprises 238 amino acids (MID962-1200). In the present paper, we show that a recombinant fusion protein consisting of MID962-1200 linked to EGFP specifically bound to human B cells. MID962-1200 stimulated (increased thymidine incorporation) peripheral blood lymphocytes 5- and 15-fold at 0.1 and 1.0 μg/mL, respectively. In the presence of T cell cytokines, MID962-1200 activated B cells. Whole M. catarrhalis bacteria strongly stimulated B cells, whereas mutated strains devoid of MID showed a 75% decreased activation. To determine the capacity of MID962-1200 to induce class switch in the presence of T cell cytokines, total RNA was isolated from pure B cells using MagnaPure technology. MID962-1200 induced mature IgG1, IgG2, IgG3, and IgA1 transcripts as revealed by RT-PCR.
Chemokines

OP31
Expression of CC chemokine receptor (CCR)11 in pulmonary sarcoidosis
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We have previously reported overexpression of a T-lymphocyte attractant,
CC chemokine ligand (CCL)19, and of its CC chemokine receptor
(CCR)7 in pulmonary sarcoidosis, a granulomatous disease with typical
CD4+ T-cell alveolitis. CCL19 has been recently shown to interact also
with CCR11. We have therefore investigated expression of mRNA for this
novel receptor in bronchoalveolar cells from patients with pulmonary
sarcoidosis and in control subjects. CCR11 mRNA transcripts were
semiquantified by RT-PCR in bronchoalveolar cells from 28 sarcoid
patients and 9 controls. CCR11 mRNA was up-regulated in patients (S)
compared to controls (C), Mean ± SEM: C, 0.44 ± 0.04; S, 0.76 ± 0.09;
P = 0.02. Subanalysis revealed a relationship between CCR11 mRNA and
chest X-ray (CXR) stage: the highest number of CCR11 transcripts was
observed in patients with the most advanced CXR stage III by contrast to
lower expression in patients with initial stage I (L, 0.69 ± 0.09; II, 0.74 ± 0.20; III, 1.04 ± 0.20). Up-regulation of CCR11 mRNA in sarcoid
lung, including observed parallel with disease course, is consistent with
overexpression of its chemokine ligand and implicates CCR11 as a
candidate for studies of sarcoidosis pathological mechanisms.

OP33
Topical glucocorticoid therapy induces CXCR4
up-regulation on primed ocular lymphocytes
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CXCR4 and its ligand CXCL12 have been reported to mediate lympho-
cyte accumulation at sites of chronic inflammation. Yet, glucocorti-
coids (GC), effective anti-inflammatory agents, have been shown to
up-regulate CXCR4 on lymphocytes in vitro. We have addressed this
paradox in the context of intraocular inflammation (uveitis). Ocular
primed T cells from uveitis patients on GC treatment expressed elevated
levels of CXCR4. This was reproduced in vitro, when CD4+ T cells were
incubated with AqH. Untreated uveitis and noninflammatory AqH
induced CXCR4 to a limited extent; this was TGFβ-dependent. The
highest levels of CXCR4, were found in GC-treated patients. GC receptor
antagonist RU486 abrogated the ability of AqH from these patients to up-
regulate CXCR4. All GC eye drops induced CXCR4 on primed CD4+ T
cells. CXCR4 was the only chemokine receptor to be upregulated by
dexamethasone. CXCL12 was found in noninflamed AqH, with very low
levels in untreated uveitis and undetectable in GC-treated uveitis AqH.
Our data suggest that CXCR4 up-regulation is a function of steroid
therapy rather than inflammation. CXCR4 induced by GC, in the absence
of CXCL12, may contribute to the resolution of inflammation.

OP32
Chemotaxis of mononuclear phagocytes toward
apoptotic Burkitt’s lymphoma cells
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Burkitt’s lymphoma (BL) contains many macrophages diffusely distrib-
uted within the tumour in a characteristic ‘starry sky’ pattern. Recruitment
of murine mononuclear phagocytes to human BL in SCID mice was
observed with significant infiltration of f4/80 positive cells into the
tumour. Using a transmigration assay we show that monocytes and
macrophages (but not neutrophils), migrate towards BL and that che-
motaxis is increased by the induction of apoptosis. Chemotaxis is corre-
lated to the number of apoptotic cells measured by Annexin V staining.
Macrophage movement towards BL cells was reduced when BL lines
were transfected with bel-2, which prevented BL-cell apoptosis. We
investigated whether macrophage receptors important in clearance of
apoptotic cells have a role in chemotaxis towards the dying cell. We saw
that although human macrophages up-regulate CD14 when migrating
towards apoptotic cells, macrophages from CD14 knockout-mice have no
defect in chemotaxis towards apoptotic cells in vitro. These results
indicate that apoptotic BL cells are likely to play a role in recruiting
macrophages to BL via mechanisms that are independent of macrophage
CD14.

OP34
Loss of CD-86 on peripheral blood dendritic cells (DC)
precedes the development of colorectal cancer
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DCs are professional apcs that play a role in antitumour immune
responses. Familial Adenomatous Polyposis (FAP) is a dominantly
inherited condition that inevitably leads to colorectal cancer (CRC)
untreated. We hypothesized that early changes in DCs may predispose
to CRC development. The number and activation status of peripheral
blood DCs in FAP and CRC patients was determined using a whole blood
assay and multicolour flow cytometry. DCs were identified as an HLA
DR + Lin- (CD3, 14, 16, 19, 34 and 56) population. The absolute number
of DCs per microlitre blood was assessed by reference to a known number
of flow count beads, and the surface expression of CD80 and 86 calculated
using the Win List programme. The number of Lin-DR + putative DCs per
microlitre whole blood was not reduced in FAP or CRC patients. Interest-
ingly, there was a significant reduction in the number of CD86 positive
cells in both patient groups (P < 0.009) and of CD 80 positive cells in CRC
patients (P < 0.05). In conclusion the total number of circulating DCs is
preserved in FAP and CRC patients. However there is a reduction of CD
86 in FAP and CRC patients suggesting that loss of maturation markers
occurs early in tumourogenesis.
OP35  
**Strain-dependent leukocyte trafficking in a murine model of allergic airways disease**

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Discrepancies between findings in different murine models of allergic airways disease may be explained by strain-dependent variations. The aim of this study was to investigate leukocyte trafficking and resolution of inflammation in different strains of mice during allergen-induced lung inflammation. Leukocyte numbers in the lung, airway lumen and draining lymph nodes were assessed at 24 h and 1 week after ovalbumin challenge in sensitized Balb/c and C57BL/6 mice. At 24 h the C57BL/6 mice had higher numbers of Th2 cells and eosinophils in the airway lumen than the Balb/c mice, while in the lung, the opposite was true. Similar levels of AHR were seen between strains, while serum IgE levels were 3-fold higher in Balb/c mice. In the C57BL/6 mice, inflammation had resolved in the lung a week after the final antigen challenge, although Th2 cells were present in the lymph nodes. However, in Balb/c mice Th2 cells but not eosinophils persisted in the lung and airway lumen. In conclusion, there are strain dependent differences in the trafficking of leukocytes between lung compartments and resolution of inflammation. Further studies are ongoing to examine the mechanisms involved.

OP36  
**Evaluating functional responses in chemokine antagonists at an early stage of the drug discovery process**

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Hit To Lead (HtL) within AstraZeneca is a crucial step within the drug discovery pipeline. HtL is the process where we take compounds with confirmed activity and synthesize related compounds to explore whether these compounds are robust leads for a major lead optimization project to develop a candidate drug. Having more confidence in the quality of drug leads at the start of a major project saves us time and money in avoiding high attrition rates. As part of the HtL process we have often been limited in studying functional responses with a wide range of compounds in any series simply due to the limitations of certain technologies. We now want to add more functional biological data as early as possible by bringing functional assays into the screening cascade sooner than we have previously. Flow Cytometry in 96 well plates is used to deliver data from integrin up-regulation assays and cell shape change in whole blood assays. Chemotaxis is now a fully automated assay in our hands and this has lead to a much more robust and less noisy assay. Automation is employed within all of our assays, so as to keep the assays standardized and ensure high replicate quality.

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**Comparative immunology**

OP38  
**Effect of tryptophan on the oxidative stress in heterophils from ring dove**


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We have found that melanotin acts as an antioxidant in phagocytic cells in *Streptococcus roseogriseus*. Here, we have studied the effect of the administration of one daily oral dose (125 mg/kg bw) of L-tryptophan (precursor of melanotin) on anion superoxide and lipid peroxide levels. Tryptophan was administered for seven days at 20:00 hours, and blood extractions to value heterophil oxidative stress after inert particles ingestion (latex beads) were carried out at 21:00 hours and 02:00 hours. Techniques used were NBT reduction test and a commercial liperoxidation kit. We observed a significative (P < 0.05) decrease halfway along the treatment in superoxide anion levels of heterophils extracted at 02:00 hours with respect to the control group, and at the end of the treatment at 02:00 hours with respect to their control group and to their respective extraction group at 21:00 hours. Besides, results showed a decrease in liperoxidation halfway along and at the end of the treatment. In the presence of the agent to phagocyte, a decrease halfway along the treatment occurs, compared to the control group. We conclude that L-tryptophan administration (125 mg/kg bw) reduces in heterophils the oxidative stress associated with phagocytic process.

OP39  
**The effect of pig genotype on acute phase protein levels**

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In addition to infection, pig genotype could also affect acute phase protein release and hence alter the immune response. We measured the effect of breed and prior genetic selection on acute phase protein levels in pigs. To achieve this aim, we measured alpha-1 acid glycoprotein (AGP) and haptoglobin in Large White pigs selected for high lean growth (n = 31), low lean growth (n = 38), high food intake (n = 24) and low food intake (n = 26). Pigs were tested at age 18 and 24 weeks. AGP and haptoglobin levels were then measured in 20 Large White pigs and 20 Meishan pigs at age 18 weeks. Pigs from either divergent breeds or lines selected for the same criterion were tested contemporaneously. For selection effects, high lean growth pigs had higher AGP levels than low lean growth pigs at age 18 weeks (631 v.363 µg/mL, P < 0.01) and age 24 weeks (315 v.215 µg/mL, p < 0.05). Selection for food intake did not affect AGP or haptoglobin levels. For breed effects, Large White pigs had higher AGP levels than Meishan pigs (405 v.260 µg/mL, P < 0.05). Neither breed nor selection for lean growth affected haptoglobin levels. To conclude, differences in pig genotype were shown to influence AGP levels but did not affect haptoglobin levels.
Cloning of bovine CD89 and localization of the IgA binding site


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We have cloned, sequenced and characterized an IgA Fc receptor from cattle. By screening a translated EST database with the protein sequence of the human IgA Fc receptor (hCD89) we identified a putative bovine homologue (bCD89). COS-1 cells transfected with the putative bCD89 cDNA bound both bovine and human IgA, but not bovine IgG2 nor human IgG1. bCD89 was thus confirmed to represent a bovine IgA Fc receptor. The bCD89 cDNA is 873 nucleotides long and is predicted to encode a 269-amino-acid transmembrane glycoprotein composed of two Ig-like extracellular domains, a transmembrane region, and a short cytoplasmic tail devoid of known signaling motifs. Genetically, bCD89 is more closely related to hCD89, bFcγ2R, and the KIR and LILR gene families than to other FeRls. Like hCD89, the bCD89 gene maps to the leukocyte receptor complex close to the KIR gene cluster. In addition, we have identified specific residues within the EC1 domain of bCD89 which are essential for binding to IgA. Further characterization of bCD89 will aid in the understanding of IgA–CD89 interactions, and may facilitate the identification of CD89-like IgA receptors from other species.

Cytokines

Study of inflammatory markers; Interleukin-6 (IL-6) and C-reactive protein (CRP), and cardiac enzymes in acute myocardial infarction (AMI): use of troponin T (TnT) concentrations as an indicator for the myocardial infarct size

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Objectives To study plasma levels of IL-6 and CRP and to assess accuracy of measurement of TnT concentration 72 h after MI for estimation of infarct size if compared to thallium 201 and other cardiac enzymes with and without early reperfusion.

Design 30 patients with recent MI have been recruited in the study for assessment of IL-6, CRP and cardiac enzymes including TnT during the first 5 days and thallium 201 scintigraphy within 10–20 days after MI.

Results The size of MI assessed by thallium 201 uptake has shown a linear correlation with TnT concentrations as well as mean concentrations of CK, CK-MB and LDH. The inflammatory markers were high for 5 days after MI and normalize within 4 weeks.

Conclusion Single measurement of TnT 72h after onset of chest pain is considered as a golden parameter or infarct size independent of reperfusion. It does not show significant correlation with IL-6. We also conclude that inflammation and myocardial damage caused by AMI are diminished within 4 weeks after onset.

Assessment of circulating tumour necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and C-reactive protein (CRP) in obese and nonobese patients with type-2 diabetes mellitus (DM)

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Aim The goal of this study was to find out the role of adipocyte-derived cytokines in insulin resistance and to determine whether inflammatory markers have a role in development of type-2 DM.

Participants 60 patients with known type-2 DM, 30 out of them were obese, have been recruited for assessment of serum concentrations of TNF-α, IL-6, and CRP and correlated with 60 age and sex matched healthy nondiabetic volunteers.

Results IL-6 was significantly higher in diabetics, particularly obese patients with obvious insulin resistance, than control subjects. TNF-α levels were greater in obese than nonobese diabetics, but did not show significant differences between diabetics and nondiabetic healthy volunteers. Higher CRP levels were detected in diabetic patients than controls, but did not show significant differences between obese and nonobese patients.

Conclusion obesity has a remarkable role in development of insulin resistance as well as overt diabetes through release of fat cell-derived mediators like TNF-α and IL-6. Elevated IL-6 and CRP as pro-inflammatory markers support a possible role for inflammation in diabetogenesis.

Cytokine phenotype of allergen activated lymph node cells (LNC)

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Topical exposure of BALB/c strain mice to the contact allergen 2,4-dinitrochlorobenzene (DNCB), or to the respiratory allergen trimellitic anhydride (TMA) induces selective type 1 or type 2 cytokine secretion profiles, respectively. The contributions of CD4 and CD8 cells to these cytokine phenotypes have been investigated using flow cytometry for intracellular cytokine staining. Results from three independent experiments are displayed. An increased frequency of IL-4+CD4+ LNC was recorded following TMA exposure compared with DNCB treatment (2.7% ± 0.5 and 0.7% ± 0.4, respectively). In all LNC populations less than 0.1% of CD8+LNC expressed IL-4. Exposure to both TMA and DNCB resulted in marked increases in IFN-γ+ CD8+ cells (56.2% ± 9 and 59.1% ± 6, respectively), compared with vehicle-treated controls (25.1% ± 8). In all populations, less than 3% CD4+ LNC expressed detectable IFN-γ. Thus, topical exposure of mice to either class of chemical allergen results in increased numbers of IFN-γ+ CD8+ cells, but treatment only with TMA is associated with an elevated frequency of IL-4+ CD4+ cells. The vigorous IFN-γ production by DNCB-activated LNC is likely to be regulated at the level of secretion.
OP44
Monocytes expressing transmembrane TNF up-regulate endothelial cell adhesion molecules
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During sepsis, blood monocytes are sequestered in the microcirculation in close contact with endothelial cells. We previously found in an in vivo model that monocytes sequestered in the lung of LPS-injected mice express transmembrane TNF (tmTNF). Here we investigated whether blood monocytes can up-regulate endothelial cell adhesion molecule (CAM) via a contact-dependent, tmTNF-mediated event in an in vitro model of leukocyte-endothelial cell interaction. LPS × induced-up-regulation of surface CAM (E-selectin, VCAM-1 and ICAM-1 by flow cytometry) on b.End5 cells was enhanced by coculture with blood leukocytes from C57BL6 mice. At a low LPS dose, this effect was reversed by an anti-TNF antibody, and expression of tmTNF was detected on monocytes for several hours. CAM up-regulation was still present after addition of a metalloprotease inhibitor (BB94), despite the absence of detectable soluble TNF. Physical separation of b.End5 cells from LPS-prestimulated leukocytes by inserts demonstrated that cell contact was essential for CAM up-regulation with BB94. These results provide the first direct evidence that murine blood monocytes are capable of activating endothelial cells via tmTNF-mediated signaling.

OP45
The relation between cytokines and VEGF polymorphisms in acute transplant rejection
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Introduction VEGF is a growth factor that increases the production of inflammatory cytokines and vice versa, while anti-inflammatory cytokines inhibit VEGF production. Previous studies have shown the association between acute cardiac and heart transplant rejection and two polymorphic sites within the VEGF promoter (G-1154 A and C-2578 A).

Objective To investigate the relation between cytokines and VEGF polymorphisms in kidney and heart transplant rejection.

Methods Production of VEGF by LPS stimulated PBMCs has been studied in presence and absence of TNFα and IL-4. VEGF in supernatant of cultured PBMCs was measured by ELISA. VEGF promoter polymorphisms were assayed by ARMS-PCR.

Results PBMC carrying the –1154 G/G and –2578 C/C genotype could produce significantly more VEGF. Our results showed, although TNFα increase and IL-4 decrease VEGF production, but the influence of these cytokines was not related to genotype.

Conclusion Promoter region of VEGF is highly polymorphic and two of these were associated with acute kidney and heart transplant rejection. However, our study showed no association between those polymorphic sites and enhancement or suppression of VEGF in presence of cytokines.

OP46
Quantitative real-time RT-PCR for the measurement of feline cytokine mRNA expression in skin
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Feline allergic skin disease is associated with a cellular infiltrate belonging to the Th2 subset and associated cytokines. Real-time RT-PCR assays were developed to measure feline interleukin-2 (IL-2), IL-4, IL-5, IL-6, IL-10, IL-12 (p35 and p40), IL-18, tumour necrosis factor-alpha, interferon-gamma and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA in lesional and nonlesional feline skin. Total RNA was extracted using the RNeasy Mini Kit with on-column and in solution DNase digestion steps. cDNA was synthesized using Improm-II reverse transcriptase and random hexamers. Real-time PCR was carried out using an iCycler IQ system (Bio-rad), and gene-specific primers designed to span an intron/exon junction of each cytokine gene. SYBR Green I was used to optimize each assay and to obtain a melting temperature of the PCR product. Taq-Man probes were subsequently used to add specificity to the system. Messenger RNA from the housekeeping gene GAPDH, which was readily detectable in nonlesional skin biopsies, was used for normalization of the cytokine threshold cycle. The assays are highly sensitive and permit quantitative analysis of cytokine mRNA expression in feline skin biopsies.

OP47
Regulation of IL-3 gene expression in Cd34+ progenitor cells in CML
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Chronic Myelogenous Leukaemia (CML) is mediated by the BCR-ABL oncogene and excessive proliferation of CD34+ myeloid progenitor cells. Growth of CML progenitor cells is dependent on the BCR-ABL induced expression of the autocrine growth factor IL-3. We are investigating mechanisms of IL-3 gene regulation by normal pathways and by BCR-ABL. We identified a conserved region 4.5kb upstream of IL-3 gene which contains an inducible DNase Hypersensitive (DH) site. A 245-bp fragment spanning this DH site acts as an inducible enhancer. This enhancer is induced by kinase and calcium signalling pathways and is activated via several binding sites for NFAT, AP-1, Sp1, GATA and c-Myb proteins. Activation of these sites may regulate expression of IL-3 in both normal cells and CD34+CML cells. In addition, we are also investigating a constitutive DH site 4.1kb upstream of the IL-3 gene in CML CD34+ cells which includes a STAT site that may also mediate activation by BCR-ABL. The aim of this study is to is to use in vivo footprinting to identify which regulatory elements are occupied by transcription factors in CML cells as compared to normal cells before and after stimulation.
OP48
RT-PCR analysis of healthy human blood shows significant diurnal variation in IL-8 but not other inflammatory mediators

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A study comprising of 16 healthy human volunteers was conducted to determine whether cytokine profiles in healthy individuals fluctuate over the course of a day. Blood samples were taken from each volunteer at 8 am and 6 pm to determine whether any diurnal variation existed. mRNA was extracted using Roche magnetic separation technology and cytokine profiles were analysed using real time RT-PCR. A range of inflammatory mediators were investigated including the pro-inflammatory cytokines IL-1β, IL-6 and TNF-α, anti-inflammatory IL-10, chemokactants IL-8 and MCP-1 and the marker of apoptosis Fas-L. Results indicate a significant reduction in the expression of IL-8 at 6 pm when compared to 8 am, but no diurnal variation was observed for any of the other inflammatory mediators investigated. This suggests that in the absence of infection the expression of IL-8 changes in response to diurnal factors.

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Subcutaneous footpad injection with a single dose of N. brasiliensis excretory/secretory (NES) antigen in complete Freund’s adjuvant (CFA) induces IL-4 production by peripheral lymph node cells. As NES clearly is able to generate a Th2 response in the presence of microbial stimuli (e.g. Mycobacterial cell components present in CFA), it is an example of a ‘dominant’ Th2 stimulus, which contrasts with many of the current models of the Th2 response. Ex vivo intracellular cytokine staining indicates that the initial production (72 h post injection) of NES/CFA dependent IL-4 in the local draining lymph node is derived from CD3+CD4+ T cells, is independent of IL-4R signalling and is concurrent with a decrease in the levels of IFNγ and IL-10 producing CD4+ cells. More recent work has concentrated on the ability of NES to influence the differentiation of nonparasite specific transgenic CD4+ T cells in vivo.

OP51
Cytokine and chemokine expression by human follicular dendritic cells

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The molecular basis for the functions of human follicular dendritic cell (FDC) is unclear. It is important to know what cytokines and chemokines are expressed by FDC. We have established an FDC cell line that has characteristics of human FDC. The FDCs were isolated by positive selection using a monoclonal antibody (H2) with a magnetic cell separation system following the initial culture of tonsillar adherent low-density cells. Cells have been shown by immunofluorescence and flow-cytometry to express ICAM1, CD40 and VCAM1 and are stained positive by H2 monoclonal antibody. In an in vitro cell culture model we have shown that stimulation of tonsillar B cells with a CpG oligodeoxynucleotides (ODN) in the presence of FDCs induces significant production of IgM, IgG and IgA. Cytokine and chemokine protein expression by these FDCs were analysed by a 79-human cytokine protein array. The FDCs expressed a variety of cytokine and chemokines including IL-6, IL-8, MCP-1, MIP-1beta, TGFbeta, IP-10, LIF, IGFBP3, TIMP-1 and TIMP-2 were also strongly expressed. The expression of these cytokines and chemokines by FDCs may be important in GC development and in supporting B cell survival and differentiation.

OP52
Cytokine responses in serious pneumococcal infection

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The pattern of cytokine response in serious pneumococcal infection in humans is unknown. Serum samples were collected from six patients at the same time as pneumococcal serotypes S3, S4, S6A, S9V, S18 and S19F were isolated from blood culture. Ten cytokines (IL1, IL2, IL4, IL6, IL8, IL10, IL12, TNF-alpha, INF-gamma and GM-CSF) were measured using a coated-bead flow cytometric method. Raised IL2 and IL10 concentrations were found in all patients. The patient infected with the virulent S3 serotype, had increased concentrations of all cytokines except IL12 and GM-CSF; two deceased patients also had increased concentrations of most cytokines. The two younger patients, infected by S4 and S19F, had lower cytokine concentrations than the older patients. Further investigation on a larger sample is needed to ascertain whether the observed variations are determined by pneumococcal serotype, or individual difference such as patient age. Variable cytokine responses in serious pneumococcal infection could be valuable in guiding treatment and evaluating patient outcome.

OP50
Early in vivo events in Th2 differentiation and development induced by excretory/secretory products of Nippostrongylus brasiliensis

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The rodent gastro-intestinal nematode parasite Nippostrongylus brasiliensis induces a CD4+ T cell dependent Th2 response in its host.
Gene therapy

OP53
Gene transfer leads to increased MHC class I expression and immunogenicity of the murine Colon-26 cell line
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In gene therapy approaches, transfected tumour cells are used to test the efficacy of different transgenes. We are working with the Colon-26 (C26) cell line, which has a heterogenous expression of H-2D, present on ~15–50% of the cells. However, stably transfected lines C26-CD40L, C26-GFP, and C26-hygroycin expressed H-2D on ~100% of the cells. In vitro experiments showed an up to 60% enrichment of H-2D+ cells after transient transfection, and antibiotic selection lead to the production of H-2D+ cell lines. In vaccination experiments none of the transfectants grew in vivo, and protection against a challenge of C26 cells was seen in 46% (C26-CD40L), 56% (C26-GFP) and 63% (C26-hygro) of cases. We then selected H-2D positive cells from the C26 cell line. C26-H-2D+ cells grew in 50% of mice. Where tumours formed, tumour growth regressed or was retarded compared to growth of wildtype cells. Our results show that the up-regulation of MHC class I through gene transfer can partially contribute to the immunogenicity of stable tumour cell transfectants, and that the presence of the hygroycin resistance construct itself is sufficient to induce an antitumour immune response.

HIV

OP54
Protection of HIV-1-specific T cells by early treatment of acute infection
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We hypothesize that functional cellular immune responses induced immediately following HIV-1 infection may be preserved by early treatment with HAART during acute disease. Thus allowing eventual discontinuation of drug therapy and induction of long-term nonprogressor (LTNP) status. We have monitored the quality and breadth of the CD4+ and CD8+ T cell responses in 7 patients, with early stage HIV-1 disease, beginning HAART for a 60-week period. Responses were measured by both proliferation and intracellular cytokine staining as we have shown previously discordance between proliferative responses and cytokine production. Results show that there is a peak of IFN-γ production to HIV-1 Tat, Nef and Gag by week 12. Both responses detected by proliferation and IFN-γ production were maintained throughout the period studied. We conclude that HIV-1-specific T cell functionality is protected by early treatment during acute infection, as previously suggested by Walker and colleagues, however, the maintenance of these responses has yet to be assessed in the long term follow-up.

OP55
Comparison of circulating levels of IL-7 in HIV2 and HIV1 infected patients
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IL-7 is thought to be a potent modulator of T cell homeostasis, able to restore immune competence through a combination of increased thymic output and enhanced peripheral expansion of T cells. Clinical studies have reported an increase in circulating levels of IL-7 in the settings of HIV1 induced T cell depletion, idiopathic CD4 lymphopenia or following cancer chemotherapy. Here, we investigate the role of IL-7 in T cell homeostasis through the comparison of HIV2 and HIV1 diseases, which are known to be associated with markedly different rates of CD4 decline. An ultra sensitive ELISA was used to quantify serum IL-7 in 43 HIV2 and 55 HIV1 therapy naive patients and 26 healthy controls. A significant increase in circulating IL-7 was documented in the HIV2 cohort, which negatively correlated with total CD4 and naive CD4 T cell levels. Worth noting, for a given degree of CD4 depletion, serum IL-7 was no higher in the HIV2 cohort than in the HIV1 infected patients. In conclusion, IL-7 appears to be an important factor in T cell homeostasis in HIV2 disease. Moreover, these data suggest that the increased rate of CD4 loss in HIV1 infection is due to factors other than the IL-7 mediated homeostatic response.

OP56
Cellular immune responses to mycobacterial antigens in HIV infection
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Background: Diagnosis of tuberculosis is difficult in patients with HIV coinfection. An accurate diagnostic test for Mycobacterium tuberculosis (Mt) infection would facilitate control of TB among HIV coinfected individuals. ESAT-6 and CFP-10 are antigens expressed in Mt but not in M. bovis or most environmental mycobacteria. We investigated the use of an ex-vivo ELISPOT assay to identify T cells specific for these antigens in HIV patients.

Methods: Ex-vivo ELISPOT assays were performed for IFNγ using ESAT-6 and CFP-10 derived peptides, PPD and PHA on peripheral blood mononuclear cells.

Results: Among HIV+ patients, 77% responded to ESAT-6 and 79% responded to CFP-10 by ELISPOT. This compares to ESAT-6/CFP-10 response rates of ≥79% HIV-TB cases and 25% in healthy individuals indicating little or no loss of test sensitivity in HIV infection; and the presence of active TB infection in a high proportion of HIV subjects.

Conclusion: Even with advanced HIV and low CD4 counts, low but detectable cellular responses to Mt proteins were found, but at lower levels.
early viral replication. To gain an overview of functional HIV-specific CD8+ T cell responses in primary infection, we initially used IFN-γ ELISPOT assays to measure responses to 5 HIV proteins at time-points during and after seroconversion. In patients who established high persisting viral loads, the primary HIV-specific CD8+ T cell response was typically biased towards immunodominant epitope(s) in a few viral proteins. By contrast, in patients who established low viral loads, strong CD8 responses were detected to multiple viral proteins, suggesting an association between rapid development of a broad and codominantly directed HIV-specific CD8+ T cell response and good control of early virus replication. To test this hypothesis further, we are carrying out a comprehensive analysis of the epitope breadth and relative immunodominance of responses within primary CD8+ T cell responses in acute/early infection in patients establishing high and low persisting viral loads.

Immunotherapy

One-step enrichment of leucocyte subsets in the blood collection tube

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The ideal method for isolating cell subsets from whole blood would involve a single manipulation, thus maximizing cell recovery and viability. We have therefore performed one-step RosetteSep negative cell selection directly in Cell Preparation Tubes (‘CPT’; Becton-Dickinson, USA). CPT are evacuated blood collection tubes containing ficoll below a gel insert and anticoagulant above the gel. Peripheral blood was collected into either standard collection tubes or CPT, and incubated with Rosette-Sep antibody cocktail. The blood collected into standard tubes was then diluted, layered over ficoll and centrifuged. The blood collected into CPT was simply centrifuged. Enriched cells were collected from the plasma: ficoll interface and analysed by flow cytometry. The purity and recovery of desired cells enriched with either approach was similar. Monocytes enriched with either method were cultured in GM-CSF, IL-4 and TNF-α. After 7 days, the cells lost expression of CD14 and expressed markers typical of dendritic cells. The advantages of using CPT are: (1) all procedures are performed in the collection tube; (2) layering the blood over ficoll is unnecessary; and (3) it is very easy to remove the enriched cells.

In vitro generation of PSA-specific CD8+ T cells in normals and patients with prostate cancer

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Identification of Tumour-associated antigen (TAA) derived peptides is essential for development of peptide-based cancer vaccines. We pulsed DCs with 8 HLA-A2-restricted peptides derived from Prostate Specific Antigen (PSA) and defined their optimal culture conditions with autologous HLA-A2+ T-cells for the generation of PSA-specific CTL. CMYp36S95−303 and IMP38a-66 peptides were used as controls. Binding of peptides to HLA-A2 was examined by stabilization of MHC-peptide complexes on TAP-deficient cells (T2). IFN-γ-ELISPOT assay was performed to enumerate the peptide-reactive CD8+ T cells generated. Expansion of CD8+ T cells specific for 3 of the 8 PSA peptides was observed in some normals but not in a patient with prostate cancer. Specific lysis of peptide-pulsed T2 cells, and of a Prostate Carcinoma cell line was detected in a cytotoxicity assay in the normals, but not in the patient with prostate cancer. Specific recognition of PSA peptides may thus occur in normals and may be lost in patients who have prostate cancer. This supports the concept of using PSA as a target molecule for vaccination in Prostate Cancer. Results of studies on more patients with Prostate cancer will be reported.

Generation of response to therapeutic immunization following extensive characterization of the murine model tumour E.G7-OVA

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We have observed for the first time in our hands that therapeutic immunization by particle mediated immunotherapeutic delivery (PMID) can influence growth of a solid tumour from the murine lymphoma E.G7-OVA. The tumour expresses chicken ovalbumin as a model antigen. C57BL/6 mice were implanted subcutaneously with 5 x 10⁶ live E.G7-OVA cells, then immunized on days 2 and 4 post implantation by PMID with 1.0 µg of the ovalbumin encoding plasmid pVAC1.OVA(cyt). Palpable tumour volume was recorded daily until tumours reached a maximum of 2000 mm³. The mean time taken to reach 2000 mm³ in the therapy group was 20.2 ± 0.4 days, compared with 17.3 ± 0.1 days in a control group immunized with empty vector (mean ± SEM, n = 12, P < 0.01). Previous studies have shown prophylactic, but not therapeutic, responses against E.G7-OVA using PMID, so the observed effect is seen as important. Extensive in vitro and in vivo study of E.G7-OVA has provided an understanding of changes in surface antigen level critical in generation of the observed effect. Current experiments are attempting to evaluate the potential of adjuvants and immunization schedule changes to modulate the effect.
OP61
A novel VAP-1 blocking antibody for the treatment of chronic inflammation in vitro
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Adhesion molecules expressed on endothelial cells recruit lymphocytes to areas of inflammation. The blockade of these adhesion molecules should prevent lymphocyte extravasation and reduce the majority of the pathology associated with chronic inflammation. One such molecule is VAP-1. Monoclonal antibodies to VAP-1 block the binding of lymphocytes, but there are disadvantages in the therapeutic use of conventional antibody Fc regions due to the inflammatory reactions resulting from the antibody effector functions. We have produced a number of recombinant chimaeric antibodies to the human VAP-1 antigen using modified human IgG constant region domains. The modifications in constant region G2α mean that the resulting antibodies should not activate the complement pathway and have reduced binding to Fc receptors. Antibodies using the G2α constant region have been shown to block lymphocyte adhesion to VAP-1, indicating that ligand binding has not been altered. In a group of assays designed to examine the modified effector function of the panel, they were shown to induce lower IL1 and TNF-α release in some donors, and to have reduced lytic activity in ADCC and complement assays.

OP62
Bio-availability of antitoxins and antitoxin fragments following intravenous or intranasal administration
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Prevention of disease after exposure to a biological toxin is partially a function of the immunity of the exposed individual. The only available countermeasure that can provide immediate immunity against such a biological agent is passive antibody. Unlike vaccines which require time to induce protective immunity and depend on the host’s ability to mount an immune response, passive antibody can theoretically confer protection regardless of the immune status of the host. Passive antibody therapy also has the advantages of high specificity, low toxicity and immediate action. This study determined the bio-availability of antitoxins and antitoxin fragments in order to assess the suitability of these agents as therapeutics for botulinum intoxication.

OP64
Modification of IL-7 and its use in the treatment of thymic involution
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Thymic involution is one of many events that leads to the inefficient functioning of the elderly immune system. This loss of immune function, can be in part characterized by the loss of the cytokine IL-7. Several investigators have shown that when IL-7 is administered in vivo it can enhance de novo T-cell production by the thymus, but there are several problems with giving IL-7 as a therapy: IL-7 has a short half life making it necessary to give repeated injections and it must be administered at a high enough dose to allow it to reach the thymus. An alternative approach, and one which should reduce dosage levels and treatment frequency, would be to create a fusion protein between IL-7 and a chemokine receptor whose ligand is organ-specific. The chemokine CCL25 is thought to play a major role in T-cell development as an attractant for thymocytes, which express the receptor CCR9. Thymic tissue expresses high levels of CCL25 and CCR9 shows a strict specificity for its ligand CCL25. CCR9 is therefore an ideal choice as a targeting component for IL-7. I show here the creation of a CCR9/IL-7 fusion protein, which retains its IL-7 activity and shows an increased ability to target the thymus.

OP65
Correlation of cytokine mRNA and protein levels in prostate cancer is a useful marker of immune activation
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We examined 60 patients at varying stages of prostate cancer (PC) and examined their protein and mRNA levels for six cytokines (IFNγ, TNFα, IL2, IL4, IL5 and IL10) using a novel real-time PCR reaction which may be reliably compared to absolute protein levels from the CBA. There was no statistically significant elevation in levels with disease progression, although there did appear to be an upward trend as metastases formed. In all cases the correlation between protein and mRNA was consistent. A subgroup of patients (28) were enrolled in a Phase II clinical trial using whole cell allogeneic vaccination. Those that showed a decrease in the rate of release of Prostate Specific Antigen (PSA) also showed a complete reversal of the protein and mRNA correlation. In these responding patients, extremely high levels of protein were isolated from PBL but very low levels of gene copies could be detected.

We believe that this assay may be of use in immunotherapy trials since we have now defined the range of cytokine protein release in PC and have shown that extremely high levels of protein, or very low levels of transcript, correlate with changes in an established clinical marker.
OP 65A
Generation of nonimmunogenic protein therapeutics by removal of T cell epitopes
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Interferon alpha (IFNα) has been used for the treatment of hepatitis C and some carcinomas, where it can inhibit viral replication or tumour cell growth. Clinical studies show that treatment with IFNα produces a deleterious antibody response against this therapeutic cytokine even though it consists entirely of human sequence. Sustained, high affinity anti-IFNα responses are CD4+ T-cell dependent. We therefore reasoned that immunogenicity can be eliminated by first identifying the CD4++ T cell epitopes and then second, introducing mutations that prevent peptide epitopes binding to MHC class II. Using this two-step approach (termed DelImmunization™), we have produced a nonimmunogenic IFNα by mutating specific residues clustering to three regions that contain T cell epitopes in the IFNα sequence. In vitro assays show that the lead IFNα has equivalent antiviral and antiproliferative activity compared to wild type IFNα. Furthermore preclinical immunogenicity assays using synthetic peptides spanning the three mutated regions demonstrate an inhibitory effect on human T cell proliferation. These findings validate DelImmunization™ technology as a means for improving immunogenicity profiles of therapeutic proteins.

Molecular immunology

OP66
HSP70-hom gene polymorphisms in sarcoidosis
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In the present study the association between DRB1*03 and the course of sarcoidosis was revisited to find out whether HSP70-hom gene polymorphisms could contribute to that already known association. HSP70-hom gene polymorphisms at positions +2763 (A/G) and +2437 (C/T) were analysed in 42 sarcoidosis patients, 13 of which presented with Löfgren’s syndrome. The following frequencies of HSP70-hom alleles were detected: HSP(+2763)-A: 0.381, -G: 0.619 and HSP(+2437)-C: 0.429, -T: 0.571. A strong linkage between HSP(+2763)-A and HSP(+2437)-C alleles as well as between HSP(+2763)-G and HSP(+2437)-T was observed (P = 0.004). DRB1*03 prevailed in Löfgren’s syndrome patients (P = 0.02). The presence of DRB1*03 together with HSP(+2763)-G and HSP(+2437)-T was found to associate with the development of Löfgren’s syndrome symptoms (P = 0.015).

OP67
The architectural transcription factor HMGA1 participates in interferon-gamma gene transcriptional control
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The High Mobility Group (HMG)1A is a nuclear protein found to regulate transcription of a number of immune-related genes, by functioning as an architectural factor to (1) modify chromatin structure (2) to bind to AT-rich sequences thereby modulating local DNA conformational change and loading of transcription factors (3) to mediate interaction between transcription factors in formation of a multiprotein complex (enhancosome) on target gene promoters. Assembly/disassembly of HMG1A-dependent enhancosomes plays a key role in controlling the rate and level of transcription. We have generated transgenic mice expressing HMG1A from the distal lck promoter/enhancer. Direct studies of T cells isolated from these mice indicate an up-regulation of interferon-gamma produc-

tion. In parallel studies in vitro, we observe an elevated interferon-gamma promoter activity in transiently transfected EL4 cells overexpressing HMG1A. In vitro binding assays demonstrate a specific interaction of HMG1A to specific regions of the interferon-gamma promoter. Taken together, our data suggest that HMG1A positively controls interferon-gamma expression at the transcriptional level.

OP68
Transcriptomic analysis of human CD4/CD25 regulatory T cells
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Naturally occurring CD4/CD25+ T cells (Tregs) play an essential role in peripheral T cell-mediated regulation. Compelling evidence has demonstrated their pivotal role in maintaining natural self-tolerance and regulating the immune response to foreign antigens. Given the contact dependent function of Tregs, we aim to identify specific transcripts encoding cell surface molecules by high-density cDNA microarray technology. Linear amplification of mRNAs has been employed and the derived cDNAs were hybridized on Affymetrix arrays representing 12,000 genes. The gene expression pattern of Tregs was overall very similar compared to CD4/ CD25− T cells, despite their major functional differences. We have identified several sets of genes differentially expressed in human Tregs. Of special interest are adhesion molecules and chemokines receptors that could reveal distinct trafficking patterns. GPI-anchored proteins and G-protein coupled receptors that may as well influence lipid raft organization and signalling events. We are in the process of validating and screening at protein level several of these candidate genes that could lead to the identification of specific molecular events in the Tregs.

OP69
The GM-CSF enhancer functions via NFAT binding and chromatin remodelling
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An inducible transcriptional enhancer exists 3kb upstream of the GM-CSF gene and is active in a wide range of cell types, such as T-cells and
myeloid cells. Changes in chromatin structure within the enhancer, as assessed by formation of a DNase I hypersensitive (DH) site, are also inducible and correlate with enhancer function in all cell lines examined. Transient transfection assays with an enhancer mutated at putative transcription factor binding sites and linked to a GM-CSF promoter and reporter gene allowed us to identify two composite NFAT/AP-1 sites, on adjacent nucleosomes, that are essential for normal enhancer function \textit{in vitro}. \textit{In vivo} footprinting of human T lymphoblasts revealed the composite NFAT/AP-1 sites are occupied within 30 min of cell stimulation. Sites within the enhancer for the constitutively expressed factors AML1 and Sp1 also only became occupied after stimulation. The occupation of the transcription factor sites and formation of the DH site occur prior to the induction of GM-CSF transcription. These findings support our hypothesis that NFAT mediated chromatin remodelling of the enhancer is necessary for the accessibility of Sp1 and AML1 and subsequent GM-CSF enhancer function.

**OP70**

The membrane proteins CD200 and its receptor CD200R interact through their membrane distal Ig-like domains

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CD200 is a broadly distributed cell surface glycoprotein that interacts with a receptor (CD200R) that is restricted to cells of the myeloid lineage and is involved in modulating macrophage activity. Both these proteins are type 1 membrane glycoproteins belonging to the immunoglobulin superfamily containing two extracellular Ig-like domains, a membrane distal V domain and a membrane proximal C2 domain. Previous studies have already implicated the V domain of CD200 as the domain that interacts with CD200R. We show that the CD200 binding site on CD200R is on the V domain GFC face. Thus the CD200–CD200R interaction spans four Ig domains, a distance of about 15 nm, which is similar to the distance spanned by many other cell surface protein interactions, e.g. those between T cells and antigen presenting cells (the immunological synapse). Similar contacts seem likely between myeloid cells (CD200R) and a variety of other cells (CD200).

**OP71**

Proteomics analysis of human CD4/CD25$^+$ regulatory T cells

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Naturally occurring CD4/CD25$^+$ T cells or regulatory T cells (Tregs) engage in the maintenance of immunological self-tolerance by actively suppressing self-reactive lymphocytes. The molecular properties that characterize Tregs remain elusive. We are embarked on the definition of proteins that are specifically expressed in human Tregs and are potential candidates for mediating suppression. We are comparing the proteomes of human CD4/CD25$^+$ and CD4/CD25$^+$ T cells by two-dimensional electrophoresis (2-DE) followed by protein identification by mass spectrometry (MALDI-MS) and database searching. The pattern of protein distribution was compared using ImageMaster 2D Elite software. An average 450 protein spots have been detected in each population and characterized in terms of their isoelectric points, molecular weight. Approximately 140 proteins were similar in both populations. 237 protein spots were only present in Tregs and 274 protein spots only detected in CD4/CD25$^+$ cells. 27 spots showed a 2 fold-increase and 64 spots showed 0.5 fold-decreased when comparing CD4/CD25$^+$ T respect to CD4/CD25$^+$ T cells. Protein spots identified by MALDI-MS analysis are being further evaluated for their relevance in the function of Tregs.

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**Neuroimmunology**

**OP72**

Follicular dendritic cell de-differentiation dramatically reduces scrapie susceptibility

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Transmissible spongiform encephalopathies (TSEs) may be acquired peripherally, in which case infectivity usually accumulates in lymphoid tissues before spreading to the brain. Studies of mouse scrapie models have shown that follicular dendritic cells (FDCs) are critical for scrapie accumulation in lymphoid tissues and subsequent neuroinvasion. Treatment with a lymphotxin β receptor (LTβR) fusion protein temporarily de-differentiates FDCs by blocking maturation signals from B cells. Here, a single treatment with LTβR-Ig before intraperitoneal scrapie inoculation blocked the early accumulation of scrapie in the spleen and significantly reduced disease susceptibility. Single treatments up to 6 weeks after scrapie challenge result in progressively smaller but significant delays in neuroinvasion. In contrast to results following ip challenge, LTβR-Ig treatment shortly before oral scrapie challenge prevents transmission, while treatment 2 weeks after oral challenge has no effect. While manipulation of FDCs may offer a potential approach for early intervention in peripherally acquired TSEs, these data suggest the duration of the treatment window may vary widely depending on the route of exposure.

**OP73**

Modulation of Fas – ligand expression in the barrier epithelia of the eye and brain by inflammation

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This study investigated the distribution of Fas-L, hr44, and CD63 in the barrier epithelia of the eye and brain. We have shown that hr44 and CD63, components of exosomes, are lost from the ciliary epithelium of inflamed eyes. Immunohistochemistry for CD63, hr44 and Fas-L was conducted on normal and mildly inflamed rat eyes and brains to determine if Fas-L immunoreactivity behaved in a similar fashion. Fas-ligand immunoreactivity was particularly prominent in the ciliary epithelium, the choroid plexus epithelium, and ependymal cells. In mildly inflamed rat eyes Fas-L immunoreactivity was lost from the ciliary epithelium. Regression analysis of staining intensity for hr44 and Fas-L indicated a very strong correlation ($P = 0.0001$). Fas-L can be shed from the ciliary epithelium and possibly the barrier epithelia of the brain following incorporation into exosomes. Findings also indicate that Fas-L expres-
sion may be inducible in the pigment epithelium of the eye in response to inflammatory stimuli. We suggest that the release of Fas-L bearing exosomes by barrier epithelia is an innate mechanism for the control of inflammation in immune privileged sites.

OP74
Smokers express significantly higher levels of the dopamine receptor subtypes D3–D5 on eosinophil populations compared with nonsmokers

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Nicotine has been shown to suppress antigen presentation by dendritic cells, and activation of T-helper cells. Dopamine may also influence immune function. Eosinophils were purified by density centrifugation and negative immunomagnetic bead selection using anti-CD16 and the dopamine receptors expressed on eosinophils were identified using flow cytometry with subtype-specific antibodies. Eosinophils have moderate but variable expression of the receptor subtypes. Dopamine receptors D3 and D5 were found on eosinophil populations of most individuals whereas D2 and D4 had more variable expression. D1 was never found. These results document expression of different dopamine receptors on human eosinophils from both smokers and nonsmokers. Smokers had significantly higher levels of expression of D3, D4 and D5 receptor subtypes. Also the culture of purified peripheral lymphocytes from nonsmokers with varying concentrations of nicotine altered the levels of expression of the dopamine receptor subtypes. This presents a possible mechanism by which smoking can affect the immune system and the interaction between the cholinergic and dopaminergic systems.

OP75
The association of serum vascular endothelial growth factor (VEGF) and interleukin-10 (IL-10) in brain tumours

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A prospective analysis was undertaken to measure levels of VEGF-A and IL-10 preoperatively in patients with brain tumours. Serum samples were obtained from 27 patients prior to surgery and also from tumour cyst fluid in 2 cases. VEGF-A and IL-10 levels were measured by quantitative ELISA. Patients were divided into low grade (3 patients), high grade (15 patients) and metastatic (9 patients) groups. The low grade tumours had a mean serum VEGF-A level of 256 (SE ± 32) pg/mL; high grade 388 (SE ± 51) pg/mL; and metastatic group 479 (SE ± 72) pg/mL. Tumour cyst fluid contained VEGF-A at 16 960 pg/mL in a benign tumour and 230 160 pg/mL in a malignant tumour. Serum IL-10 was only detectable in 6 patients at 3.1 (SE ± 0.6) pg/mL and there was no association between this cytokine and tumour grade. There appears to be an increase in serum VEGF-A levels as tumour grade increases with the highest levels being seen in patients with metastatic tumours. The significance of the approximately thousand-fold increase of VEGF-A in cyst fluid as compared with serum levels needs further analysis in a larger series of patients. The results of this pilot study indicate no association between serum VEGF-A and IL-10.

OP76
Dysregulation of adaptive immunity in Guillain–Barré Syndrome

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Guillain-Barré Syndrome (GBS) is a monophasic acute inflammatory process affecting peripheral nerves and their roots, considered autoimmune in origin. An animal model of GBS is mediated by T cell responses against myelin antigens. There is conflicting evidence of adaptive immune responses against myelin proteins in GBS patients. Whole and parts of candidate autoantigens myelin protein zero and peripheral myelin protein 22 were used in ELISA and Western blot studies with serum from 37 GBS patients and healthy controls. Serum responses to these myelin proteins were found in 20% of GBS patients. We conclude that B cell responses against these proteins cannot fully explain the pathogenesis of GBS in our patient cohort. We studied circulating lymphocytes by flow cytometry in 21 GBS patients and 20 healthy controls. The CD4/CD25+ HELAD+ T cell population was reduced acutely in GBS patients, tending to return towards control levels after intravenous immunoglobulin. This suggests a reduction in regulatory T cells or migration of cells away from the peripheral circulation in GBS. Whether loss of regulatory T cells is important in the pathogenesis of GBS deserves further exploration.

Parasitology

OP77
Evidence for the direct recruitment of eosinophils by the ectoparasite Psoroptes ovis

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The mite, Psoroptes ovis, is the causal agent of sheep scab, an allergic dermatitis associated with a rapid inflammatory response dominated by the mass influx of eosinophils. Current work is based on the hypothesis that P. ovis may contribute directly to the recruitment of eosinophils, which are the predominant cells of the definitive lesion. Initial invitro studies showed that whole mite extracts from different donor sources contained significant and dose-dependent chemotactic activity for sheep bone marrow eosinophils. Subsequently, live mites incorporated directly into the chemotaxis procedure were also shown to evoke a significant eosinophil chemotactic response suggesting the presence of secreted and/ or excreted chemotactic product(s). More recent evidence indicates that P. ovis mites maintained on agar plates produce an excretory/secretory exudate, which also induces a potent eosinophilic effect. These
results confirm that *P. ovis* mites contain and/or release factors that are chemoattract for eosinophils *in vitro*. This reinforces our contention that *P. ovis* may directly contribute to the recruitment of eosinophils *in vivo* and this could have important implications for pathogenesis and control.

**OP78**

**Immune-mediated control of intestinal epithelial cell turnover during infection with *Trichuris muris*: a new mechanism of worm expulsion?**

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It is well established that the development of a Th2 response is necessary for the expulsion of *T. muris* from the intestine, although the effector mechanisms responsible for expulsion remain to be determined. It is clear that the expulsion of intestinal nematodes can involve the interaction between CD4+ T cells and the gut epithelium. As the intestinal epithelium is under constant renewal, we hypothesized that an elevation in the rate of epithelial cell turnover may mediate worm expulsion. To examine this we have looked at epithelial cell kinetics in resistant (BALB/c, IL-4 KO female) and susceptible (AKR, IL-13 KO and IL-4 KO male) mice during infection. Results demonstrate that upon infection, the rate of epithelial cell turnover is elevated. This increase is almost twice as great in resistant mice compared to susceptible mice. These findings suggest that an increase in the rate of epithelial cell turnover may be responsible for worm expulsion. We propose that this novel mechanism of worm expulsion is mediated by the generation of Th2 cytokines by CD4+ T cells within the intestinal environment.

**OP79**

**MHC, antigen recognition and resistance to nematodes**

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The nematode *Teladorsagia circumcincta* is a serious constraint on livestock production. A major manifestation of resistance in growing lambs is reduced adult worm size and fecundity. Lambs with shorter worms have more parasite-specific local IgA and they recognize a different set of parasite molecules than their susceptible contemporaries. Variation in antigen recognition is associated with particular polymorphisms at the class II MHC DRB1 locus. Lambs that are more resistant to nematode infection have alleles that are associated with recognition of ‘protective’ parasite molecules.

**OP79A**

**Characterization of the immune response against the three isolates of *Trichuris muris***

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The murine intestine dwelling parasite, *Trichuris muris* is used as a model for the human disease caused by *T. trichiura*. Three isolates of *T. muris* have been identified, E, J and S. They differ in expulsion kinetics and the immune response elicited in the host. Many laboratory mouse strains, such as NIH and BALB/C, are resistant to the E isolate, producing a strong Th1 helper (Th2) response resulting in expulsion of the worms. Susceptible strains of mouse, for example AKR, produce a Th1 response to infection with the E isolate allowing persistence of infection. However, the slower responding C57BL/6 mouse strain produces a predominantly Th2 response to the J isolate, a mixed Th1/Th2 response to the E isolate and little or no response to the S isolate of *T. muris*. This was characterized by very low production of the cytokines, IL-4, IL-13, IL-5, IL-9 and IL-12 in mesenteric lymph nodes of mice infected with S. This leads to investigations into early processing of antigen from the three isolates by dendritic cells and antigen recognition by IgG1 and IgG2a from infected animals. Data from these experiments are presented.

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**Rheumatology**

**OP80**

**Interleukin-18 promoter polymorphisms associated with rheumatoid arthritis (RA)**

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**Introduction**

IL-18 in RA promotes inflammation via innate and adaptive immune responses. Promoter polymorphisms may modulate IL-18 expression. Using two independent clinical cohorts, we determined the frequency of single nucleotide polymorphisms (SNPs) in the IL-18 promoter of RA patients.

**Methods**

IL-18 SNP was determined by either RFLP or allele-specific PCR.

**Results**

Frankfurt: 640C (*P* = 0.005) and −170C alleles (*P* = 0.028), and −640CC and −170CC diplotypes (*P* < 0.001) were significantly more frequent in the RA population. Linkage disequilibrium however, was only found for RA patients at position −170.

**Glasgow**

The −170C allele (*P* = 0.03) and −170CC diplotype was more frequent in RA patients (*P* = 0.02). In addition, the −170CC and CG genotypes were significantly more frequent in RA (*P* = 0.005). Again, allelic distribution of RA patients at position −170 was in disequilibrium.

**Conclusion**

SNPs at the −170 position in the IL-18 promoter appear to contribute to the genetic background in RA pathogenesis. Importantly, this has been independently identified in two clinical cohorts. IL-18 is a promising therapeutic target and, as such, defining factors that modulate its regulation in rheumatoid tissues is important.
OP81
TNF-α up-regulates in vivo homing of a synovial specific phage to synovial grafts in the human/SCID mouse transplantation model

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It has been previously demonstrated that in vivo selection of phage display library in synovial tissue transplanted SCID mice can identify displayed peptides sequences that home specifically to the human microvasculature endothelium in these grafts. One such clone, 3.1 phage clone displaying the sequence CKSTHDRLC, has shown consistent synovial specific homing in this model. The aim of this work was to study the effect on homing capacity of the 3.1 phage clone to transplanted tissues injected with TNF-α. It was shown in this study that in mice transplanted with human RA synovial and normal skin tissues, intragraft injections with TNF-α (200 ng/transplant) caused a significant increase in the number of 3.1 phage homing only to the synovial graft after 6 h of stimulation. Furthermore, the number of 3.1 phage diminish back to the level of the saline control after 24 h. Although these results are not direct evidence of cellular adhesion molecule (CAM) involvement, it does show that the up regulation expression of the synovial specific counter-ligand for the 3.1 phage clone is similar to the time course of TNF-α up-regulation of CAMs such as E-selectin.

OP82
Spontaneous inflammatory arthritis and dermatitis in thymectomized, CD25− depleted adult mice

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This study investigated the effect of CD25− cell depletion on collagen-induced and spontaneous arthritis. Male DBA/1 J mice (5/group) were treated with 1 mg ip anti-CD25 depleting antibody or isotype control, either early (days 1, 6 and 14) or late (days 14, 21 and 28) relative to immunization with bovine collagen type II emulsified in CFA. Separately, adult male DBA/1 J mice were thymectomized and treated with anti-CD25 antibody or isotype control (12/group). The phenotype of CD25+ cells in peripheral blood was monitored by FACS analysis. The emergence of arthritis was monitored by an arthritis severity score. In nonthymectomized mice, anti-CD25 Ab had no significant difference in the natural history of arthritis. In the thymectomized mice treated with anti-CD25 Ab, spontaneous arthritis and dermatitis occurred 6 weeks after depletion (arthritis severity score elevated; P < 0.01). This model reveals a role for CD25+ constitutive cells in suppressing inflammatory disease in this arthritis-susceptible mouse strain.

OP83
IL-18 in Sjogren Syndrome (SS): a serological and immunohistochemical (IHC) study

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SS is an autoimmune disease (AD) characterized by periductal mononuclear cell (MC) infiltration of lachrymal and salivary glands (SG). Evidence suggests that SS is a Th1-mediated AD but cytokine(s) driving the Th1 response in SS have not been characterized. IL18 plays a pivotal proinflammatory role in several Th1-mediated AD. We studied 37 sera and 10 SG biopsies from patients with SS. Serum IL18, anti-Ro, anti-La and anti-Ro/SSA antibodies (Abs) were measured by ELISA. IL18 expression in SG biopsies was evaluated by standard and double IHC. Serum IL18 levels were increased in SS compared to controls. Anti-Ro and anti-La+ve patients showed higher serum IL18 compared to anti-Ro and anti-La–ve and a positive correlation between IL18 and the titer of anti-Ro, anti-La and IgA antifodrin Abs was observed. IL18 expression in SGs was observed in 29 out of 32 MC foci and was colocalized with CD68+ cells. IL18 was also expressed by CD68+ macrophages in ectopic germinal centers (GC). Production of IL18 was also found in ductal epithelial cells (DEC). Detection of increased IL18 in serum, correlation with serum autoAbs and localization in MC foci, ectopic GCs and DECs suggests a prominent role for IL18 in SS.

OP84
PKC-eta expression and IL-1 activation involved in NOS II induction in human peripheral blood monocytes of severe inflammatory arthritis patients

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Patients with moderate to severe inflammatory arthritis (IA) (more than 5 effused joints) expressed NOS II on their PBM and had elevated plasma NO levels, unlike healthy controls, patients with mild IA, or osteoarthritis. Conventional treatments had no effect on NOS II expression or plasma NO levels. Infliximab (anti-TNF-alpha) or anakinra (IL-1Ra) treatments of severe IA patients were both effective in reducing symptoms, but only anakinra reversed the NOS II positive phenotype and restored the plasma NO levels close to normal. PKC-eta was always expressed in PBM from moderate or severe IA patients but was never present in controls. PKC-eta was usually coexpressed with NOS II or under some circumstances expressed alone. Absence of PKC-eta appeared to preclude NOS II expression. These data allowed us to hypothesize that a PKC-eta positive phenotype may exist prior to the induction of NOS II, and that IL-1, either directly or indirectly, is responsible for the development of the NOS II phenotype in PBM. It was further concluded that neither TNF-alpha nor IL-1 appear to be responsible for the development of the PKC-eta phenotype.
Transplantation

OP85
A linkage association study in the multidrug resistance gene (MDR-1)

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Background The closely spaced single nucleotide polymorphisms frequently yields highly correlated to extensive linkage disequilibrium (LD). The multidrug resistance (MDR-1) gene’s product is also an important molecule regulating the bioavailability of immunosuppressive drugs.

Objective To develop genotyping assays for polymorphisms of the MDR-1 gene that are believed to have functional properties.

Methods Eight polymorphisms in the MDR-1 gene were selected for analysis (G-41A, G-145C, C-129T, T1236C, G2677A, G2956A, C3435T, C4030G and G4036A). Genotype assays performed by using different PCR techniques in UK Caucasoid.

Results A variant A-allele at 2677 tended to be accompanied by the C-allele at 3435 as compared with a variant T-allele.

Conclusion Two polymorphisms in exon 22 (A1a to Thr) and exon 26 (silent) of the MDR-1 gene were linked in our subjects. This finding suggests the importance of haplotype assignment for the MDR-1 gene. The polymorphism C3435T in the MDR-1 gene has shown to correlate with low levels of gene expression. It is possible that variant alleles present in the MDR-1 gene of an individual may influence their response to immunosuppressive therapy.

OP86
Visualization of peptide-specific alloreactive T cells using HLA class I tetramers

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An explanation for the vigour of alloreactive T cell responses may be that numerous antigenic targets are created by the array of peptides presented by HLA molecules. We sought to confirm this using HLA class I tetramers to identify the ligands recognized by alloreactive T cells. A panel of A'0201/peptide tetramers was generated with self-peptides known to bind to A’0201. Alloreactive CD8+ T cell lines were propagated using A’0201 transfectants of the LCL 721.221 or A’0201 dendritic cells. Peptide specific alloreactive T cells were propagated by preincubating T2 cells with each peptide. T cell lines specific for A’0201 were screened for tetramer binding and cells that recognized each HLA/peptide alloantigen were quantified. For all HLA/peptide combinations tested a small population of tetramer binding CD8+ T cells was found. Lines stimulated using peptide pulsed T2 cells exhibited exquisite specificity. Our results provide evidence that the vigorous alloreactive T cell response is caused by the summation of numerous responses to each of the peptides bound by the allogeneic HLA molecules. Pooled tetramers are being used to screen for alloreactive T cells in HLA mismatched transplant patients.

OP87
Porcine CTLA4-Ig as a specific reagent to prevent direct pathway xenospecific T cell sensitization

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The inhibition of the delivery of costimulatory signals is an attractive therapeutic strategy to prevent rejection in organ transplantation. For this purpose, we have constructed a fusion protein consisting of the extra-cellular regions of pCTLA4 and the constant regions of human IgG1 (pCTLA4-Ig), which failed to inhibit costimulation provided by human B7. The aim of this study was to confirm the species specificity in the action of pCTLA4-Ig in a mouse experimental model. pCTLA4-Ig bound poorly to murine CD80 and CD86 expressed on transfectants, and gave minimal staining of the CD80+ murine cell line DAP3 and to mature dendritic cells. pCTLA4-Ig blocked mouse T cell responses to pig but not mouse dendritic cells. We have also tested the efficacy of pCTLA4-Ig at preventing pancreatic islet rejection. Mice injected with pCTLA4-Ig exhibited prolonged islet survival (36.5 ± 9.4 days, n = 6) compared to controls treated with isotype antibody (9.8 ± 2.9 days, n = 6). Our results indicate that pCTLA4-Ig is a relatively specific inhibitor of the direct mouse T cell response to porcine tissues and therefore, is a potentially important therapeutic reagent to use in clinical xenotransplantation.

OP88
Kinetics of circulating dendritic cell subpopulations in chronic myeloid leukaemia patients after reduced intensity allogeneic stem cell transplant

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Dendritic cells (DC) are key components of the immune response, but if derived from malignant monocyte precursors in vitro show defects in phenotype or function. This may not reflect the situation in vivo. Circulating monocytoind (mDC) and plasmacytoid (pDC) DC in peripheral blood (PB) from patients with CML (n = 8) were compared with normal subjects (n = 12). PB was taken at diagnosis, after Glivec therapy and post allogeneic stem cell transplant (SCT). DCs were quantified and expressed as percentage/white blood cells (WBC) and absolute numbers/mL. In normal subjects, mDC = 0.45% (1.45 × 103/mL) and pDC = 0.24% (1.04 × 103/mL). At diagnosis, CML patients had reduced DC levels (mDC = 1.3 × 103/mL/pDC = 0.51 × 103/mL). After treatment, mDC increased slightly and pDC were elevated significantly (0.32%, p = 0.045). At 72–100 days post SCT a massive increase in mDC occurred both in percentageWBC (P = 0.006) and absolute numbers (40.23 × 103/mL, P = 0.047). The mDC/pDC ratio (10.0:1) was skewed towards mDC compared with normal/diagnosis values. The massive increase in mDC after allogeneic SCT may relate to successful engraftment and functional responses to new host or tumour antigens.
OP89
Chemokine–glycosaminoglycan interaction during inflammation
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Chemokines have the potential to bind both their specific receptors and glycosaminoglycans (GAGs). In this study we investigate the significance of these interactions in regulating inflammation. Relative cell surface expression of Heparan sulphate (HS), Chondroitin sulphate (CS) CS4 and CS6 was determined using microvascular endothelial cells. Analysis of results by confocal microscopy and flow cytometry showed that HS was most abundant followed by CS4 and CS6. In response to stimulation with pro-inflammatory cytokines binding of the chemokine RANTES to cell surface GAGs increased significantly. The specificity of this interaction was verified by pretreatment with heparitinase. Paraffin-embedded normal and rejecting renal biopsy tissue were stained for GAGs and RANTES: rejecting biopsies had increased HS followed by CS4 and CS6. HS staining was restricted to the basolateral domain and basement membrane of the tubular epithelium whereas the CS showed more extracellular matrix staining. Dual immunofluorescence demonstrated that RANTES colocalized with HS stained areas. Thus increased expression of cell surface GAGs during rejection may provide a regulatory mechanism during inflammation.

OP90
Enumeration of alloreactive T cells with indirect allospecificity using MHC class II tetramers
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The development of an effective in vitro assay capable of predicting the likelihood of graft survival after organ transplantation has become the focus of much attention. The ultimate goal is to employ an assay which is able to detect a state of tolerance in transplant patients. Here we describe a novel approach to enumerate T cells with indirect allospecificity using MHC class II tetramers. Due to the technical difficulties in generating MHC class II tetramers, they, unlike their class I counterparts have had limited use in monitoring T cell responses and have never been tested in the context of indirect allore cognition. We have generated an indirect alloreactive T cell line which is specific for HLA-A*0201 (103–120) in the context of HLA-DRB1*0101. Using MHC class II tetramers we were able to visualize these indirect allospecific T cells. A 28-fold increase of CD3+CD4+ ‘tetramer’ cells was seen in the alloreactive T cell line compared to unstimulated autologous PBMCs. The clinical utility of this approach in the detection of CD4+ T cells with indirect allospecificity for HLA-A2 in DRB1*0101 ‘A*0201’ transplant recipients is currently underway.

TP91
Copper induction of beta-sheet conformation in the ovine prion protein
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Prion diseases are characterized by conformational change in the copper binding protein PrP. Polymorphisms in ovine PrP protein at amino acid residues 136, 154 and 171 are associated with variation in susceptibility to scrapie. V136R154Q171 (PrP-VRQ) or A136R154Q171 (PrP-AQRQ) animals show susceptibility to scrapie whilst those that express A136R154R171 (PrP-ARR) show resistance. We have investigated the stability and effects of metal ion-interaction on the structure of ovine recombinant PrP-VRQ and PrP-ARR protein. Alpha-helical PrP-VRQ was thermodynamically more stable than PrP-ARR and displayed a different dimeric configuration. Copper was a cofactor in the conversion of alpha-helical ovine PrP to the beta-sheet form. However, copper-treated PrP-VRQ showed a greater degree of beta-sheet conformation than did PrP-ARR, although both allelic forms acquired resistance to Protease K digestion following exposure to this metal. Copper-induced conformational changes occurred in the C-terminal portion of ovine PrP as evidenced by modulation of anti-PrP monoclonal antibody epitopes in this region of the molecule. We suggest that allelic variants of ovine PrP differ in their structure and response to copper.

OP92
Conformational mapping of cell surface ovine prion protein
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Amino acid residue 171 of the ovine prion protein plays a critical role in determining the susceptibility of sheep to natural scrapie. The conformation of ovine PrP will be influenced by the particular amino acid located at this site. Variation in the conformation of cell surface PrP on peripheral blood mononuclear cells from VRQ/VRQ, ARQ/ARQ or ARR/ARR homozygous sheep was analysed by FACS using a panel of anti-PrP monoclonal antibodies. Cells from all three genotypes appeared to express similar levels of PrPC when assessed using an N-terminal specific antibody. Monoclonal antibodies that bind around helix-1 revealed structural heterogeneity between the three genotypes of PrP. The region of PrPC between the second beta-strand and helix-2 was found to be inaccessible to monoclonal antibodies that react with an epitope that includes amino acid residue 171. Collectively, these findings suggest that different allelic forms of cell surface ovine PrP have a common N-terminus structure, display different conformations in the C-terminal portion of the molecule and that the region around amino acid 171 is normally buried within the molecule.
OP93
Immunophenotyping and PrP detection in lymph nodes of scrapie challenged sheep
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Scrapie is a member of the transmissible spongiform encephalopathy family and affects both sheep and goats. The abnormal form of prion protein (PrPSc) is a marker used to detect disease in the animal. Serial kill pathogenesis studies have shown that the lymphoreticular system is one of the first sites of PrPSc accumulation prior to involvement of the CNS. In this study, 12 New Zealand Suffolk scrapie free sheep were challenged with natural scrapie brain by subcutaneous injection in the drainage area of the prefemoral lymph node. Challenged and contra lateral lymph nodes were surgically removed in two phases (early and late) which are 2 weeks and 6 months post challenge, respectively. Two normal brain inoculated animals were used as controls. Using immunohistochemical studies, we observed that after 2 weeks post challenge there was a marked increase of secondary follicles and PrP accumulation in follicular dendritic cells (FDCs) in the challenged lymph nodes. Flow cytometric studies revealed an increase in the CD21 population at 6 months post challenge.

Tumour immunology

OP95
Study of hepatocyte growth factor (HGF) in Hodgkin (HL) and non-Hodgkin lymphomas (NHL)
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Aim To compare serum levels of HGF in HL and NHL and to correlate its levels to clinical and histopathological grading.
Design Serum levels of HGF have been measured in 38 patients with lymphomas (10 patients with HL and 28 with NHL), and 20 sex and age matched healthy controls. All patients were subjected for clinical staging and histopathological grading.
Results HGF levels were significantly higher in lymphoma patients if compared to healthy controls. HGF showed higher levels in patients with advanced clinical stages, those with apparent B-symptoms, and those with high international prognostic index (IPI). No significant differences were detected between HL and NHL or on basis of histopathological grading.
Conclusion Although HGF levels could be considered as an indicator for disease widespread and more clinical impact, it was not related to degree of proliferation or histopathological grading. Obviously, HGF may have no pathogenic role, but can be used as a prognostic tool. We will extend our study by measuring HGF expression in the tissues which will be more conclusive.

OP96
Prediction of HLA-DR7 binding peptides from the Wilms’ tumour antigen and use of dendritic cells to generate CD4+ WT1-specific T cells in the Saudi population
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The Wilms’ tumour (WT1) gene performs an oncogenic function in leukaemia and is known to be overexpressed in several leukaemias therefore making it a suitable target for immunotherapy. Our PCR analysis showed an aberrant expression of WT1 gene in the Saudi leukaemia patients (3/3 ALL, 3/3 AML) but not in normal subjects (0/3 B-MLC). WT1-specific CD8+ T cells restricted to HLA class I has been identified. However, it is known that CD4+ helper T cells restricted to HLA class II binding epitopes are required for optimum CD8+ T cell efficacy. We observed that screening of the WT1 protein sequence against the SYFPEITHI computer algorithm, resulted in 10 peptides predicted to bind highly to the HLA-DRB1*0701 molecule (the most common allele in the Saudi population). Further, we optimized a dendritic cells (DCs) protocol that yields highly activated mature DCs [CD1a = 98%, CD83 = 82%, CD80 = 98%, CD86 = 98%, HLA-DR = 100%]. T cells are being sensitized with DCs-pulsed with these peptides. Further these T cells will be tested against B-MLC pulsed-peptides and/or transfected with WT1 cDNA, and against HLA-DR7-matched patients’ leukaemic cells.
**OP97**

**Phenotypic and functional analysis of CTLs Melan-A specific from healthy donors in vitro stimulated**


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Melan-A26-35 is an epitope recognized by CTLs melanoma specific. The aims of this work has been to analyse the phenotype and function of CTLs Melan-A specific in healthy donors as well as determine the inhibition-activation cytotoxicity balance mediated by NK receptors and costimulatory molecules. The study of costimulatory molecules on resting and in vitro expanded CTLs Melan-A specific from healthy donors showed a high expression of CD27 and CD28, moreover, we found that NK receptor expression is very low in both situations. The phenotypic analysis of activation/differentiation markers on CTLs Melan-A specific showed a naive phenotype (CD45RA+CCR7+) which changes to an effector/memory phenotype (CD45RA+CCR7-) when they are expanded with IL-2 and IL-7. The functional assays with CTLs Melan-A specific from healthy donors showed that these cells acquire IFN-gamma secretion capacity when they are in vitro stimulated, moreover, the cytotoxicity is very low before and after in vitro stimulation. In conclusion, the in vitro stimulation model allows us to determine the role of inhibitory receptors and costimulatory molecules on melanoma cell lysis mediated by specific CTLs.

**OP98**

**HLA-B35-restricted immune responses against survivin in cancer patients**

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Two HLA-A2 restricted epitopes have recently been identified from the broadly expressed tumour antigen survivin, and several vaccination trials in cancer patients based on these survivin-derived peptides have been initiated. Consequently, there is a crucial need for the identification of survivin epitopes restricted to other HLA-molecules, to extend the proportion of patients that can enter these ongoing clinical trials. In the present study we characterized two survivin derived epitopes, which are restricted to HLA-B35. Specific T-cell reactivity against these survivin-derived epitopes was found in the peripheral blood from patients with different B-cell malignancies and melanoma. Substitution of the C-terminal anchor residue of the survivin-derived peptides, improved the recognition by tumour infiltrating lymphocytes from melanoma patients. Furthermore, we demonstrated spontaneous cytotoxic T-cell responses to survivin in a primary melanoma lesion. The characterization of these epitopes allows more patients can be included in the ongoing peptide-based survivin vaccination trials against cancer.

**OP99**

**Expression of MHC class I-related molecule MICA in breast carcinomas and its prognostic significance**

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Stress-inducible MICA, a distant homologue of major histocompatibility complex (MHC class I), functions as an antigen for gamma delta T cells and is frequently expressed in normal intestinal epithelium and many carcinomas of the lung, breast, kidney, ovary, prostate, and colon. In this study a polyclonal antibody was produced, which stains MICA on fixed sections. The antibody recognized a 62 kDa band on Western blot that was blocked by MICA specific peptide, indicating the specificity of antisera. This antibody was then used to investigate the prognostic significance of MICA in 291 patients with primary operable breast cancer. 95% of the breast carcinomas showed MICA reactivity, with intensity ranging from weak (23%) to strong (37%). Expression of MICA was significantly associated with tumour grade (P = 0.014), lymph node stage (P = 0.022), Nottingham Prognostic Index (0.043) and type of tumours (0.035). The result of this study support the hypothesis that an induced expression of MICA may be a prognostic indicator for breast carcinoma and could play a role in immune responses against tumours.

**OP100**

**Complement regulatory protein CD55 as a therapeutic target for a cancer vaccine**


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CD55 is one of several complement regulatory proteins (CRPs) involved in cellular protection from autologous complement ‘bystander’ attack. Expression of the CRPs has been shown to be deregulated in a variety of tumours with CD55 being up-regulated up to 100 times normal levels. This has been identified as a significant mechanism for evading immune effectors by tumours and their microenvironment. A cancer vaccine specifically targeting this associated antigen could be used to promote existing humoral responses as well as inducing cytotoxic cellular mechanisms for tumour therapy. We have identified both CD4+ and CD8+ epitopes within the complement regulatory domains of CD55. CD4+ epitopes have been confirmed in PBMC proliferative assays and epitope specific CTL killing was obtained by repetitive in vitro stimulation of PBMC with CD8+ peptides. Mutations of HLA anchor residues have been incorporated into the epitope sequences of CD55 DNA constructs currently being assessed in transgenic mice as tumour therapeutics. These results suggest the presence of a T cell repertoire specific for CD55. This provides a novel approach for the development of a CD55 vaccine.

**OP101**

**Dendritic cell (DC) subpopulations in human peripheral lymph nodes: Studies in breast cancer and controls**

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DCs are heterogeneous, but little is known of diversity of DCs in normal and cancer draining lymph nodes. Therefore, we studied DCs from nodes draining breast cancer (n = 12), control cervical (n = 2) and inguinal nodes (n = 4). DC subpopulations and their innate cytokine production (IL-4, 10, 12 and IFN-γ) were determined by flow cytometry using off-line compensation and subtraction (Winlist, Verity). DCs were identified as lineage− and HLA-DR+, and putative subpopulations simply defined from staining profiles with CD1c and HLA-DR. Peripheral migratory cells, Lin−CD11c−HLA-DR−/−, labelled strongly CD40, CD80, CD86, and CD83. CD1a was also on this population in inguinal/axillary, but not
cervical nodes. Myeloid cells, Lin−CD11c+ HLA-DR+, were CD40 and CD86 positive, with CD80 only in advanced breast cancer (n = 6). Plasmacytoid cells, Lin−CD11c+ HLA-DR+ CD123+, were also CD40 and CD86 positive but labelled less strongly than myeloid cells. Plasmacytoid cells were major cytokine producers in all nodes. Thus, plasmacytoid, myeloid, and peripheral migratory DC may be identified in lymph nodes, with plasmacytoid DCs possibly the major source of active cytokine production.

## Accessory molecules

**OP102**  
Expression of B7 molecules by normal and malignant human uro-epithelial cells  
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Although many tumours are capable of immune evasion, the mechanism(s) involved are unclear. Epithelial cells are the origin of most adult human solid cancers and therefore understanding how they interact with the immune system is important for developing effective antitumour strategies. Because normal human uro-epithelial (NUH) cells have the ability to inhibit mitogen- or antigen-driven T cell activation, we investigated expression of the B7 family costimulatory molecules by NUH cells and their malignant (TCC) counterparts. Neither normal nor TCC-derived RT4, RT112 or EJ cells expressed B7.1 or B7.2 by RT-PCR or by flow cytometry. However, NUH cells expressed B7-H1 and B7-DC, known ligands for the inhibitory receptor programmed death-1 (PD-1) by RT-PCR and flow cytometry, and message for B7-H4. By contrast, TCC-derived cell lines expressed no B7-DC and B7-H4, and little or no B7-H1 at either message or protein levels. These results imply that normal cells express ligands for inhibitory receptors for T cell activation, which paradoxically appear to be down-regulated or lost after malignant transformation.

## Antigen presentation

**OP103**  
The ABC transporter signature motif is required for peptide translocation but not peptide binding by TAP  
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The transporter associated with antigen processing (TAP) translocates peptides into the lumen of the endoplasmic reticulum (ER) where they bind nascent major histocompatibility complex (MHC) class I molecules. Peptide binding promotes the folding and assembly of stable MHC class I molecules which are then able to traffic from the ER to the cell surface. TAP is a member of the ATP binding cassette (ABC) transporter family whose members utilize energy from ATP hydrolysis to translocate substrates across membranes. The highly conserved nucleotide binding domains (NBDs) of ABC transporters couple ATP hydrolysis to substrate translocation by the membrane domains. The conserved ‘signature motif’ can be identified in the NBDs of all ABC transporters, yet relatively little is known about its function. Here we show that the introduction of mutations into the signature motifs of either TAP1 or TAP2 inhibits the translocation of peptide without affecting binding of either peptide or ATP by TAP. We therefore conclude that the signature motifs in both TAP1 and TAP2 are required after peptide binding to facilitate peptide translocation by TAP.

**OP104**  
Development of a library screening approach to identify class II minor histocompatibility (H) antigens involved in graft vs. host disease (GvHD)  
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In HLA-identical stem cell transplantation, the desirable graft vs. leukaemia (GvL) effect mediated by donor lymphocytes is often associated with GvHD. Several minor H antigen-specific CD4+ T cell clones have been generated from skin biopsy lesions of patients with GvHD. These include an HLA DQ 0501-restricted clone, specific for a minor H epitope (HY) encoded by the Y chromosome gene DBY, and a DP 0401-restricted clone, reactive with an unknown autosomal antigen, which we will identify using cDNA expression library screening. We have used bacterially expressed DBY to optimize library screening conditions. Bacteria are preincubated with dendritic cells (DCs) at a ratio of 1:100 (bacteria:DCs), followed by addition of the DBY-specific T cell clone. Specific proliferation to bacteria expressing DBY diluted up to 1/100 is seen, indicating a library can be screened in pools of 100. These findings are being applied to determine the specificity of the DP 0401-restricted clone. Since preliminary data suggests this antigen is commonly expressed in DP 0401 individuals, it will be important to establish its role in GvL/GvHD.


**Expression of LMP7 allows processing of a minor histocompatibility antigen in professional and non-professional APC**

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We have analysed the processing requirements for generation of a CTL epitope derived from the male Uty gene and compared its presentation by professional and nonprofessional APC. Experiments carried out in LMP7−/− mice showed that presentation of UT246-254 is strictly LMP7 dependent in nonprofessional APC. In contrast, professional APC are capable of efficiently presenting this epitope in an LMP7−/− independent manner. We showed in vivo that Uty cross-presentation overcomes the LMP7 dependent block in presentation of the HYD8Uty epitope. Uty presentation by LMP7 deficient DC in vitro could be abrogated by depleting CD8α − DC from freshly isolated DC preparations. These results demonstrate that even in the absence of immunoproteasomes, professional APC can generate an immunoproteasome dependent epitope. The finding that expression of immunoproteasomes by nonprofessional APC restores optimal presentation of the UTY peptide suggests that up-regulation of immunoproteasomes by cyto-kines is a mechanism to ensure that nonprofessional APC can present a broader repertoire of class I peptides to match the multiple class I processing pathways operating in professional APC.

**Mannose receptor expression and function in murine dendritic cells**

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The mannose receptor (MR) is an endocytic receptor, with two lectin activities, that plays an important role in induction of immunity and tissue homeostasis. MR is widely expressed by macrophages (Mφ) while expression on dendritic cells (DCs) is more restricted. Cultured human monocyte-derived DCs and DCs in inflamed skin are MR+. Antigen (Ag) uptake via MR led to enhanced presentation to T cells by cultured human DCs. To assess the role of MR in Ag presentation we naive T cells in the mouse model, we performed in situ analysis of secondary lymphoid tissues and demonstrated the presence of MR+MHCII+ cells in parafollicular areas of peripheral lymph nodes (LNs). These cells are absent in mesenteric LNs. After LPS treatment MR labelling is exclusively enhanced in peripheral LNs. The absence of MR− parafollicular cells in mesenteric LNs and their failure to respond to LPS stimulation may reflect the ongoing process of tolerance in these tissues. In accordance with in situ studies, we have identified a population of MR+CD11c+MHCII+ cells by FACS analysis in peripheral LNs of untreated mice. We suggest that these MR+ cells could act as Ag presenting cells in vivo. The function of these MR+ cells is under assessment.

**Apoptosis**

**TRAIL receptor expression and function in normal and malignant human uro-epithelial cells**

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The TNF/TNFR families are critical mediators of cell survival in the immune system. TRAIL has been reported to induce death differentially in tumour cells. A robust in vitro system for normal human urothelial (NHU) cell culture was used to study expression, regulation and function of TRAIL-R in NHU cells vs. tumour (TCC) cell lines. Expression of TRAIL-R1-R4 was studied at protein and transcript levels and functional significance was evaluated with soluble recombinant TRAIL preparations. All TCC and NHU cell lines expressed TRAIL-R1 and R2. NHU cells alone expressed low levels of TRAIL-R4 (Dr2). TRAIL was not growth-inhibitory but induced apoptosis in all cell lines to varying degrees, with NHU cells showing least susceptibility. Cell death was related to the degree of ligand cross-linking. However, human fibroblasts were completely refractory to TRAIL, irrespective of cross-linking. These results demonstrated differential killing by TRAIL on tumour vs. normal urothelial cells, although significant killing of normal epithelial cells raises questions about the potency of soluble TRAIL preparations and their suitability for cancer therapy.

**Mechanism of CD40-mediated apoptosis of uro-epithelial cells: involvement of TRAF1 and TRAF3 proteins in the apoptotic pathway**


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CD40 ligation can result in growth inhibition of carcinoma cell lines from a variety of epithelial origins and can promote apoptosis following inhibition of protein synthesis. Using our in vitro system for normal human urothelial (NHU) cells and a panel of transitional cell carcinoma (TCC) lines, we have previously shown that cell-surface presented CD40L induces apoptosis of TCC but not normal cells, whereas soluble CD40L is growth inhibitory but does not induce killing (Bugajska et al. 2002, JNCI, 94, 1381). As members of the TRAF family have been implicated in CD40-mediated growth inhibition of epithelial cells, we investigated TRAF involvement in our system. We observed massive induction of TRAF1 and TRAF3 protein expression following coculture with 3T3CD40L fibroblasts in the CD40-positive TCC cells but not in NHU cells, when compared to coculture with control 3T3neo cells. Using siRNA technology, we further showed that both TRAF1 and TRAF3 are critical components of the apoptotic pathway. We believe this is the first demonstration of TRAF1 and TRAF3 up-regulation and direct involvement in CD40-mediated apoptosis of epithelial cells.
Cell signalling

OP109
Protease activated receptors on human basophils and KU812 cells
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Protease activated receptor (PAR) expression by human basophils has not been investigated. We have therefore examined PAR expression in purified human basophils and the basophilic cell line KU812. Human basophils were purified using the Miltenyi Negative Selection Kit. PAR mRNA was detected by RT-PCR using primers for the four PARs. The unique basophil secretory product, basogranulin, was assayed using dot blot analysis with the specific antibody, BB1. Ca\(^{2+}\) fluxes were measured using Fura-3. Inositol 1,4,5-trisphosphate (IP\(_3\)) was measured by radioreceptor binding assay. Thrombin (250 U/mL) and trypsin (10 μg/mL) elevated intracellular Ca\(^{2+}\) ([Ca\(^{2+}\)]\(_{i}\)) in KU812 cells. Selective agonist peptides, to PAR1 (TFFLLR, 100 μM) and PAR4 (AYFGKF, 300 μM) elevated [Ca\(^{2+}\)]\(_{i}\), but PAR2 agonist peptides (SLIGRL, 50 μM and SLIGKV, 100 μM) did not. PAR1 and PAR4 agonist peptides increased IP\(_3\) levels in KU812 cells. For all four PARs mRNA was detected in KU812 cells and purified human basophils. Agonist peptides failed to stimulate basogranulin release from basophils. These data demonstrate the expression of PAR1 and PAR4 by KU812 cells but stimulation of basophils with PAR1 or PAR4 agonist peptides does not induce degranulation.

OP110
Bruton’s tyrosine kinase is an important signalling mediator of TNF\(_{z}\) production in LPS-induced macrophages and in rheumatoid synovial cells
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Previously we have shown that Bruton’s tyrosine kinase (BTK) is an important mediator LPS-induced TNF\(_{z}\) in human macrophages using blood mononuclear cells (PBMC) from XLA patients (that are deficient in BTK expression) and by over expressing BTK in macrophages. Additionally, we have demonstrated that over expression of BTK increases TNF\(_{z}\) production by increasing TNF\(_{z}\) mRNA stability through a 3’UTR-dependent mechanism. BTK over expression studies also demonstrated that BTK increases LPS-induced kinase activity of p38 MAP kinase but does not affect ERK1/2 or JNK. As p38 has been implicated in mRNA stabilization, we have used luciferase reporter constructs with specific deletions in the TNF\(_{z}\) 3’UTR to demonstrate that BTK-dependent stabilization of TNF\(_{z}\) acts primarily through the 75 nucleotide AU-rich region of the UTR and requires p38 MAP kinase. Preliminary data show that BTK is involved in spontaneous TNF\(_{z}\) production by synovial cells from rheumatoid arthritis (RA) patients. Also, over expression of another BTK family kinase, BMX, showed that it can also modulate TNF\(_{z}\) production in macrophages and in RA through a similar mechanism.

Cellular immunology

OP111
The role of Notch signalling in the regulation of peripheral T cell function
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The Notch signalling pathway is highly conserved and is a crucial regulator of cell fate decisions in many developmental systems. In the immune system, the outcome of Notch signalling has far-reaching effects and has been shown to be critical in the control of T cell development, haematopoietic stem cell differentiation and in some models, the induction of peripheral tolerance. In order to fully investigate the role of Notch signalling in the context of peripheral immunity, we have generated transgenic mice with inducible expression of the Notch ligand Delta-1 under the control of the human CD2 promoter. Here we report, that after anti-CD3/CD28 stimulation in vitro, transgenic CD4\(^{+}\) T cells exhibit a highly irregular pattern of Th1 and Th2 cytokine production with a corresponding increase in Hes-1 transcript expression, as compared to wild-type controls. We propose that the Notch signalling pathway may have an important role in the regulation of peripheral T lymphocyte differentiation and could therefore be a potential therapeutic target for the manipulation of effector T cell function.

OP112
Survival of virulent Mycobacterium bovis and BCG differs in macrophages and dendritic cells
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The initial interactions of macrophages (M\(_{p}\)) and dendritic cells (DC) with mycobacteria are crucial factors in determining the outcome of infection. Human and mouse M\(_{p}\) and DC, when infected with mycobacteria, produce a response that reflects their roles in immunity. Thus, M\(_{p}\) elicit antimicrobial mechanisms for removal of bacteria and DC up-regulate expression of molecules that aid stimulation of T lymphocytes. We investigated the survival of virulent M. bovis, the causative agent of bovine TB, and avirulent M. bovis BCG labelled with a luciferase reporter gene in bovine M\(_{p}\) and DC. CD14+ monocytes were cultured with and without IL-4 and GM-CSF to derive M\(_{p}\) or DC that were then stimulated with either IFN-γ, TNF-α, IL-10 or left un-stimulated for 24 h prior to infection. At various times following infection cells were lysed and bacterial numbers estimated. Mycobacterial survival was greater in DC than M\(_{p}\), less with avirulent than virulent bacteria, and may be modified by pretreatment with cytokines. Thus, survival within the host may be altered by the type of host cell that interacts first with the bacterium and also by cytokines present in the local microenvironment.

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Clinical immunity

OP113
Pituitary-thyroid axis function and calcium-phosphate metabolism in major thalassemic patients, Hormozgan, Iran

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Endocrine gland dysfunction in the background of iron retention is a major problem in thalassemic patients. In this study, a number of 104 thalassemic patients, were compared with 107 healthy individuals. The results revealed that 86% of cases had ferritin level of greater than 1500 mg/mL. Prevalence of goiter in girls of both cases and controls were 74 and 42%, respectively (P < 0.05). T4 and FT4I levels in patients were significantly less than those in healthy group (P < 0.05), while T3 and TSH levels were not different. 47% of the patients suffered from subclinical hypothyroid (TSH > 5 and normal T4 and FT4I) and 9.1% of patients had normal TSH and deficiency in FT4I, which may be due to central hypothyroidism or Sick Euthyroid Syndrome (SES). 13.8% of the patients had abnormalities of pituitary-thyroid axis. Calcium averages in boys of both groups were significantly different (8.7 mg/dL vs. 9.2 mg/dL), but it was not different in girls. Phosphor level in boys and girls of cases was higher than in control group (P < 0.0001). Calcium level of less than 8.1 mg/dL was detected in 27.6% of patients and it was shown that risk of hypocalcemia increases as age increases (r = −0.33, P < 0.05).

OP114
Cytokine expression and regulation in leprosy skin lesions in Type 1 reactions

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Type 1 reactions are complications of borderline leprosy due to delayed type hypersensitivity. Patients present with acute inflammation of skin and nerves. These reactions are treated with prednisolone for 6 months. Macrophages are a feature of reaction skin lesions. This study investigates the expression of cytokines produced by macrophages in leprosy skin lesions, and the effect of prednisolone on the expression of these cytokines. The hypothesis that prednisolone treatment down-regulates inflammatory cytokines and up-regulates anti-inflammatory cytokines was tested. Skin biopsies were taken from patients in reaction during treatment. Expression of TNF-α, TGF-β, IL-10, IL-1 and iNOS were determined using real-time PCR and immunohistochemistry. Results show that TNF-α, IL-10 and iNOS protein is expressed at high levels during reaction, whereas TGF-β expression is low. A down-regulation of TNF-α, IL-10 and iNOS was observed after 4 weeks of treatment in most patients, indicating that prednisolone has a slow down-regulatory effect on cytokines in the skin. No cytokines were up-regulated by prednisolone in the skin. This may explain why these reactions are clinically difficult to control.

OP115
A FACS whole blood assay for patients with common variable immunodeficiency (CVID)

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Introduction Common variable immunodeficiency (CVID) is characterized by antibody deficiency and an increased susceptibility to recurrent bacterial infection. Recently a FACS based classification based on the distribution of memory B cell markers has been used to identify distinct subgroups of patients with CVID.

Objective To develop a whole blood flow cytometric assay suitable for classification of patients with CVID.

Methods Fresh EDTA blood samples (3 mL) were obtained from 9 patients with CVID, 9 healthy controls and 9 disease control patients (IgG2 deficiency, IgM deficiency and SPAD). B cell subsets were identified by flow cytometric analysis of whole blood: naive B cells (CD19+IgD+CD27-) and 2 memory B cell subpopulations (CD19+IgD-CD27+ and CD19-IgD-CD27+).

Results The median percentages of IgD-CD27+ (3.2%) and IgD-CD27+ (1.4%) memory B cells are significantly reduced in CVID patients compared with healthy controls (10.7% ± 12.5%) and disease controls (12.2% ± 11.7%). P-value < 0.004 Mann–Whitney test.

Conclusion This assay can be readily used in routine clinical practice to identify homogeneous groups of patients with CVID.

OP116
The effect of preoperative hyperoxia therapy on patients undergoing cardiopulmonary bypass (CPB)

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Major advances have been made in our understanding and application of safer technology during CPB. Also, there have been improvements in surgical techniques, anaesthetic and perioperative management, however, cerebral complications still represent a major cause of morbidity/disability following CPB. The incidence of neurological deficit, including cognitive dysfunction, ranges from 15 to 79%. An inflammatory response causing leukocyte activation is thought to be responsible, in part, for these effects. The aim of this work was to identify an inflammatory pathway associated with neurological deficit in CPB patients. In this pilot study 24 patients undergoing CPB were split randomly into 3 groups: (A) received hyperbaric oxygen preoperatively; (B) received air; and (C) had no pretreatment. A panel of markers (s-ICAM1, s-E-selectin, s-P-selectin, TNFz, VEGF, IL-6, IL-8 and CD18/CD11b) were studied prior to hyperoxia, preoperatively, 2 h and 24 h postsurgery. Expression of these markers were similar in Groups A and B, but TNFz was significantly different after surgery. However, Group C showed differences between both Group A and B in most markers studied.
OP117
Administration of recombinant IL-12 and IL-18 induces Th1 immunity but does not affect the salivary gland pathology in a model of Sjögren’s-like syndrome
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There is controversy concerning the role of Th1 type cytokines in the pathogenesis of Sjögren’s syndrome. MRL/lpr mice spontaneously develop disease resembling Sjögren’s syndrome. We have investigated the potential role of Th1 regulatory cytokines in the development of Sjögren’s-like disease in MRL/lpr mice. Four week old female lpr mice were given daily intraperitoneal injections (over a 6-week period) of recombinant murine IL-18 (500 ng/mouse/day) and recombinant murine IL-12 (50 ng/mouse/day). Control mice were injected with PBS. MRL/lpr mice treated with IL-12 and IL-18 produced significantly higher levels of serum IFN-γ and IgG2a but lower levels of IL-10 and IL-5 compared with the PBS controls. lpr mice treated IL-12 and IL-18 developed severe glomerulonephritis compared with PBS controls (P < 0.004, n = 10), but there was no significant difference in the severity of salivary gland pathology. These data therefore suggest that although IL-12 and IL-18 treatment produces a strong shift towards the Th1 cell activity, this does not have a significant effect on the induction of Sjögren’s-like syndrome in MRL/lpr mice.

OP118
Quantitative and functional analysis of peripheral blood NKT cells in coeliac disease
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Background Coeliac disease is a common chronic inflammatory intestinal disease induced by dietary wheat. Why oral tolerance is lost is unknown. NKT cells, a regulatory T cell subtype, mediate intestinal inflammation in mice and control many aspects of immune regulation. These cells recognize glycolipid presented by CD1d on intestinal epithelial cells.

Dendritic cells

OP120
Differential kinetics of Langerhans cell migration stimulated in mice by chemical contact and respiratory allergens: involvement of epidermal IL-10
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Preferential T helper (Th)1- and Th2-cell activation is observed following repeated topical exposure of BALB/c strain mice to chemical contact and respiratory allergens, respectively. Previously, we have reported that respiratory allergens, such as trimellitic anhydride (TMA), induce Langerhans cell (LC) migration with delayed kinetics compared with contact allergens, such as dinitrochlorobenzene (DNCB). We have now investigated whether IL-10, a cytokine implicated in the negative regulation of LC migration, is involved in the delayed kinetics of LC migration in response to TMA. BALB/c strain mice were exposed locally to anti-IL-10 antibody prior to treatment with chemical. Anti-IL-10 was found to enhance LC migration only in mice treated with TMA, and not DNCB. Supernatants derived from skin explants excised from mice exposed in vivo for 2–4 h to TMA, DNCB or vehicle revealed elevated IL-10 expression only in supernatants derived from TMA-exposed mice. These data suggest that early epidermal IL-10 production provoked by TMA may delay LC migration and may ultimately influence the development of a Th2 response to this chemical.
Absence of TNF-α and IL-1β-induced Langerhans’ cell (LC) migration in patients with psoriasis

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A proportion (20–30%) of LC migrates from the epidermis following intradermal injection of healthy volunteers with either TNF-α or IL-1β. To investigate the functional status of LC in patients with a range of severities of chronic plaque psoriasis, 6 healthy volunteers and 22 untreated patients were injected intradermally with various concentrations of TNF-α or IL-1β to sites identified on non-sun-exposed buttck skin, greater than 5 cm from a psoriatic plaque. Skin biopsies were taken 2 h later and epidermal LC densities were determined. LC numbers at saline-treated control sites fell within a similar range of values for both normal volunteer and patient groups. Although TNF-α and IL-1β each stimulated reductions in LC frequencies of approximately 25% in normal volunteers as reported previously, in patients with psoriasis LC failed to respond to either cytokine. In contrast, TNF-α and IL-1β each induced local erythema and a perivascular infiltrate in both psoriasis patients and controls. These results suggest that in uninvolved psoriatic skin, LC are unable to migrate in response to TNF-α or IL-1β, even though surrounding cells respond normally to these inflammatory cytokines.

Isolation and phenotypic characterization of dendritic cells from normal mouse liver

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Liver dendritic cells (DCs) are believed to play important roles in regulation of hepatic allograft acceptance. However, because of inherent difficulties in isolating adequate numbers of DCs from liver, limited information is available on the phenotype and functions of liver DCs. To address this issue, we isolated DCs from normal mouse liver using a modified procedure and described their immunophenotypes. Non-pan-enchymal cells were obtained by collagenase digestion of perfused liver fragments and density gradient centrifugation. After overnight incubation of the cell suspension, enrichment for transitantly adherent, low-density cells on a nycodenz gradient permitted the recovery of cells with distinct DC morphology (CD11c+). Flow cytometric analysis revealed that these cells were CD11c+/MHC-II+ (53%), CD11c+/CD86+ (53.5%), CD11c+/CD86+ (36%) and CD11c+/CD11b+ (45%). Our findings indicate that the purity of DCs isolated by nycodenz gradient is more than other reported methods. Considering the similar ratio of lymphoid (CD11c+/CD8α+) and myeloid (CD11c+/CD11b+) DCs in the liver, it seems that other factors such as the immaturity of liver DCs may be the reason of tolerogenicity of this organ.

In vitro generation and phenotypic and functional studies of monocyte derived dendritic cells

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In the present study we generated dendritic cells from peripheral blood monocytes of breast cancer patients and studied their immunophenotypic and functional characteristics. Plastic adherent peripheral blood mononuclear cells were cultured in the presence of GM-CSF, IL-4 and TNF-α. Morphological studies were made by light microscopy, phenotyping was carried out using flow cytometry and their capacity to stimulate mixed leukocyte reaction (MLR) by H-thymidine uptake test. After 5 days culture in the presence of GM-CSF and IL-4 adherent PBMCs appear as loosely adherent clumps or isolated floating cells with the typical dendritic morphology. Analysis of their surface markers showed that the large cells were homogeneous and expressed high levels of CD1a, CD11c, and HLA-DR and low level of CD14. Addition of TNF-α as maturation factor for 2 days caused increasing of cell size, membrane processes as well as expression of CD1a, CD83, and HLA-DR. In the functional point of view these cells were potent in stimulating allogeneic MLR (Mean SI = 20). Regarding our results the obtained dendritic cells can be used for research and active immunotherapy.

Cytokine secreting profile of resting and activated immature rat bone marrow-derived dendritic cells

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Dendritic cells (DCs) are described as being immunogenic or tolerogenic based on the release of pro-inflammatory or regulatory cytokines, respectively. Here we show that in vitro culture of bone marrow cells in the presence of rat GM-CSF (10 ng/ml) and IL-4 (5 ng/ml) induces the differentiation of DCs with an immature phenotype as indicated by their cytokine secretion profile. Day-7 DCs constitutively produced low levels of both the pro-inflammatory cytokine IL-12p40 and the regulatory cytokine IL-10. Stimulation of immature DCs with the potent DC maturation agent LPS (1 μg/ml for 24 h) not only induced high levels of IL-12p40 (mean: 224 vs. 5764 pg/ml), but also, counter-intuitively, high levels of the regulatory cytokine IL-10 (mean: 5.23 vs. 3640 pg/ml). These findings indicate that although the population of DCs used in this study have a functionally immature status and are susceptible to stimulation by LPS, activation arms them with both immunogenic and tolerogenic potential. This study was funded by the National Heart, Lung and Blood Institute, USA (HL 69726).

Delivery of stress proteins by antigen presenting cells

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Pristane induced arthritis (PIA) is a murine model of rheumatoid arthritis (RA). Protection from PIA is established by immunization with hsp65 or the immuno-dominant epitope (peptide 261–271) prior to arthritis induction and is mediated by hsp65/261–271 specific Th2 cytokines. The aim of this project is to examine the capacity of tolerogenic DCs pulsed with hsp60/65 or immunodominant epitopes to prevent PIA. The ongoing studies presented here characterize the immune responses induced by immunization with hsp60, hsp65 and related peptides into naïve or pristane treated animals. In addition, various regimes for the production of tolerogenic DCs are assessed.
**OP126**

**Expression of CD4 T cell epitopes from merozoite surface protein-1 on subsets of dendritic cells during malaria infection**

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Previous investigations have concluded that DC play a pivotal role in the development of protective immunity to malaria. We are therefore studying the contribution of subsets of splenic dendritic cells in the antigen presentation of Merozoite Surface Antigen-1 (MS-1) expressed by *P. chabaudi chabaudi*. MS-1 is expressed by malaria parasites during the blood-stage of the infection. Two MS-1 derived CD4 T cell epitopes have been characterized in BALB/c mice. The B7 epitope is located at the C-terminal region of MS-1 and is retained by the parasite as it invades the erythrocyte. The B5 epitope on the other hand, is contained within the region of the MS-1 protein that is cleaved prior to erythrocyte invasion. The ability of B7 and B5 specific T cell hybridomas to respond to different splenic subsets of DC was investigated. When DC were isolated from mice at the peak of parasitaemia, CD11c+CD8α- DC presented both B5 and B7 epitopes more efficiently than CD11c+CD8α+ DC. We are currently testing the different DC subpopulations for their ability to take up infected erythrocytes, and their ability to process and present MS-1 derived B5 and B7 epitopes.

**OP127**

**Differential expression of the chemokine receptor CCR7 on dendritic cells in human large and small intestine**


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**Introduction** The chemokine receptor CCR7 facilitates migration of dendritic cells (DC) from tissue to lymph nodes. Given the different bacterial microenvironment in the large and small intestine, we assessed whether DC in these different intestinal regions differed in their migratory potential.

**Methods** CCR7 expression on DC identified in lamina propria mono-nuclear cells extracted from control (colonic and ileal) and inflamed intestinal tissue was assessed by flow cytometry. Chemotactic responses to the ligand MIP-3β were also measured by chemotaxis assays.

**Results** CCR7 + DC were detected in ileal tissue from controls and migrated towards the ligand MIP-3β. In contrast, CCR7 was not detected on DC from control colonic tissue, but CCR7 was induced when these ‘immature’ DC were matured in vitro. However, in intestinal inflammation CCR7 was identified on colonic DC.

**Conclusions** Constitutive trafficking of immature CCR7 + DC, normally present only in the ileum, may maintain tolerance to commensal bacteria. In contrast, colonic DC, expressing CCR7 only on maturation, may be abnormally activated in intestinal inflammation.

**OP128**

**Altered mucosal distribution of dendritic cells in inflammatory bowel disease**


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**Introduction** Intestinal dendritic cells (DC) play a role in regulating the mucosal immunity and may contribute to the dysregulated immune response in inflammatory bowel disease (IBD).

**Methods** Expression of DC-SIGN, langerin, S-100, DC-LAMP and CD1a was assessed on intestinal tissue from patients with ulcerative colitis (UC) and Crohn’s disease (CD) and compared with control intestinal tissue using immunohistochemical techniques.

**Results** In healthy controls and CD, DC-SIGN+ cells were distributed throughout the mucosa. In contrast, DC-SIGN+ cells were located immediately below the epithelium in UC. In both UC and CD, there was a uniform distribution of intensely stained S-100 cells compared with control tissue in which weakly stained S-100 cells were located mainly in the muscularis mucosae. In CD, langerin+ cells were located deep in the lamina propria and the intensity of staining was weak compared with that seen in control tissue. Few cells stained with CD1a and DC-LAMP in all groups tested.

**Conclusion** Redistribution of DC with accompanying alteration in antigen sampling and cellular interactions may contribute to the dysregulated immune response in IBD.

**OP129**

**Mucine respiratory tract dendritic cells (RTDC): implications for intranasal tolerance therapy in experimental autoimmune uveoretinitis (EAU)**

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RTDCs form a contiguous subepithelial network within the naso-respiratory tract bridging innate and acquired immunity and have been implicated in nasal mucosal tolerance induction. Suppression of EAU can be achieved by a single intranasal application of autoantigen. One proposed mechanism is that the RTDC modulates T cell responses in the local drainage lymph node. We have assessed the response of RTDC to maturation signals and antigen priming, and whether retinal antigen pulsed RTDC prime T cell responses in vitro. After isolation from mucine lung, RTDC phenotype and cytokine profiles were determined in response to LPS, IL-4, TNF-alpha and/or peptide. RTDC have an immature phenotype displaying low levels of coaccessory molecules, and produce IL-12p40 and IFN-alpha. When pulsed with peptide that is known to induce EAU, RTDC are capable of driving a Th1 response. Results imply that tolerance is not dependent on specific RTDC behaviour but is likely to be as a result of their microenvironment, as well as route of antigen delivery.
OP130
Lipopolysaccharide (LPS) activation confers apoptotic potential on immature rat bone marrow-derived dendritic cells

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Dendritic cells (DCs) regulate T cell mediated immune responses via a number of mechanisms, one of which involves the apoptosis of activated T cells. This study demonstrates that LPS activated bone marrow-derived DCs induce the apoptosis of resting, syngenic splenocytes. Rat bone marrow was cultured for 7 days in the presence of rat GM-CSF (10 ng/mL) and IL-4 (5 ng/mL), and the resultant immature DCs were cultured for 24 h with LPS (1 μg/mL). Pre-treated and control DCs were washed and incubated for 24 h with freshly isolated, syngenic splenocytes. Apoptosis of splenocytes was determined by flow cytometry on the basis of Annexin V and propidium iodide (PI) staining. LPS pretreatment markedly increased the proportion of splenocytes undergoing apoptosis (Annexin V+/PI− 12.75% vs. 32.2%; P = 0.036), but had no effect on the proportion of early apoptotic (Annexin V+/PI−) or necrotic (Annexin V+/PI+) cells. These findings suggest that activated DCs have the capacity to eliminate mononuclear cell populations at early stages in developing immune responses. This study was funded by the National Heart, Lung and Blood Institute, USA (HL 69726).

OP131
Aspirin-treated DC induce hyporesponsiveness and regulatory activity in responder T cells

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Dendritic cells (DC) are the main professional antigen presenting cells of the immune system. However, recent studies have shown that immature DC (iDC) can induce tolerance, rather than activation, in responding T cells. As iDC are subject to maturation, aspirin was used to induce DC with a greater, or more stable tolerogenic potential than that of iDC. Aspirin-treated DC were shown to have reduced allostimulatory capacity, and to induce a Th1 profile of cytokine production in responding naïve T cells. Most significantly aspirin DC were shown to induce the development of regulatory cells in the responder CD4+ T cells. The mechanisms involved in the regulation were also investigated, and a combination of cell-contact-dependent mechanisms and inhibitory cytokines were involved.

OP132
Th1/Th2 equilibrium is influenced by Type I Interferons during dendritic cell stimulation

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Naive T cells recognize antigen on DC and differentiate into Th1 or Th2 cells. Type 1 interferons are a family of 14 closely related proteins. In addition to their well known antiviral properties, they have a wide range of immunological properties. Recent studies suggest that type 1 interferons have an important role to play in Th1 induction in the absence of DC. We investigated the role of type I IFNs in DC/T cell cross-talk. Human monocyte-derived DC and T cells produce type I IFN when activated. In addition, blocking the action of this IFN using a monoclonal antibody against the IFN receptor results in a significant increase in interferon gamma. This phenomenon was consistently observable in atopic individuals. Our data suggest that this increase in IFN-γ is dependent on interleukin-12 production by DC which may be itself dependent on the type 1 IFN produced. These results will further clarify the role of type I IFNs in T cell differentiation in the presence of DC as well as improve our understanding of the links between innate and adaptive immunity.

OP133
Regulation of dendritic cell interleukin-12 secretion by tumour cell necrosis

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Dendritic cells (DC) play a key role in the induction and regulation of antigen specific immunity. Whereas molecules derived from infectious pathogens stimulate DC maturation, recent studies have shown that cells dying by necrosis may also serve this function. However, the ability of necrotic cell death to modulate cytokine secretion by DC is not yet fully explored. In this study, we investigated the regulation of IL-12 secretion by monocyte-derived DC in response to parenchymal cell necrosis in an in vitro coculture model. Two tumour cell lines were induced to undergo necrosis. Both injured tumour cells tested in this study induced secretion of monomeric IL-12p40 by DC and up-regulated the co-stimulatory molecules. However, DC did not secrete IL-12p70 in response to necrotic cell death. In contrast, priming DC with necrotic cells did augment IL-12p70 secretion significantly in conjunction with CD40 cross-linking. We also demonstrated that both cellular DNA and proteins contributed to IL-12 secretion by necrotic cells. These findings indicate that necrotic cell death not only stimulates DC maturation but also enhances the secretion of the bioactive form of IL-12, the Th1 promoting cytokine.

OP134
IFN-γ regulates activation/maturation of DCs: influence on the Th1/Th2 equilibrium

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Different cytokines have crucial effects on activation/maturation of DCs and as a consequence on the Th1/Th2 equilibrium. While it is known that IFN-γ or PGE2-treated DCs induce Th1 or Th2 responses, respectively, the effects of IFN-α on DC activation and function are yet not clearly understood. In our study, IFN-α-treated DCs induced DC maturation and T cell proliferation. In addition, while culturing DCs with IFN-α alone amplified a Th0 response, the presence of suboptimal dose of LPS in the DC culture reduced the production of both IFN-γ and IL-5 by T cells. Th1/Th2 development is very much dependent on the balance between IL-12 and IL-10 produced by DCs. We found that only when DCs were treated with IFN-α alone, IL-12 production induced by CD40 cross-linking was increased compared to untreated DCs, while the levels of IL-10 were maintained. Altogether, our data suggest that IFN-α can amplify T cell response by acting on the DCs. Furthermore, during an immune response dominated by IL-10, IFN-α can further inhibit T cell responses and may induce regulatory T cells. Hence, the effect of IFN-α on DC function is dependent upon the environment within which this cytokine is acting.
Immunity to infection

OP135

**Beta-defensins kill Cryptosporidium parvum in vitro**

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Introduction Beta-defensins (β-defensins) are important effectors of innate immune system that secreted at mucosal surfaces. We have previously shown that *Cryptosporidium parvum* (*C. parvum*), a causative agent of diarrhoea in children and immuno-compromised patients, modulates the expression of β-defensins during infection. In the present study we have investigated the potential killing activity of defensins against the parasite.

Methods *C. parvum* sporozoites were exposed to recombinant human beta-defensin peptides (rhBD) – 1 and – 2 for 1 h. The viability of parasite was then determined by FACS analysis and by reproduction in intestinal epithelial cell line (CMT-93).

Results FACS analysis showed significant reduction in the viable sporozoite population in defensin treated samples. Moreover, defensin-treated sporozoites added to CMT-93 cells yielded significantly less intracellular parasitic development compared to untreated sporozoites.

Conclusions β-defensins exhibit potent killing activity against *C. parvum*. This confirms a critical role for defensins in innate immunity to *C. parvum*.

OP136

**The absence of Natural Killer T (NKT) cells protects mice from Chlamydia trachomatis Mouse Pneumonitis infection**

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NKT cells represent a unique subset of T lymphocytes that express NK cell-surface markers and γδT cell receptors specific for the non-MHC encoded, MHC class I-like CD1 protein. Their role in chlamydia immunobiology remains unclear. Using CD1 gene knockout (KO) mice, which lack NKT cells, in a murine model of pneumonia induced by *Chlamydia trachomatis* mouse pneumonitis (MoPn), we examined the role of NKT cells in the progression of chlamydial infection and immune-mediated pathology. The data show that CD1 KO mice displayed less severe body weight loss compared to Balb/c controls. CD1 KO mice also had a significantly higher clearance rate of the organism from lungs, liver, and heart. CD1 KO mice also exhibited lower MoPn-driven IL-4 production as well as lower MoPn-specific and total serum IgE compared to infected Balb/c controls. In addition, MoPn-infected CD1 KO mice displayed significantly more pronounced DTH responses upon chlamydial challenge. These data provide *in vivo* evidence for the involvement of NKT cells in a chlamydial infection model and their potential contribution to the background levels of key Th2-like cytokines during active chlamydial infection.

OP137

**IL-13 mediated effector mechanisms in Trichuris muris infection**


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*Trichuris muris* is an intestinal-dwelling nematode of mice. Inbred strains of mouse differ in their ability to expel *T. muris* from the large intestine. For example, BALB/c mice expel quickly, C57BL/6 mice expel slowly and AKR mice fail to expel. It is well established that the expulsion of *T. muris* from the mouse is mediated by a Th2 response and susceptibility to infection is mediated by a Th1 response. The Th2 response is characterized by the production of the cytokines IL-4, IL-5, IL-9 and IL-13 whilst a Th1 response is characterized by the production of the cytokines IL-12 and IFN-γ. Studies in IL-4 KO mice have demonstrated that IL-13 is particularly important in mediating resistance although the exact mechanisms of expulsion are still not known. Principal work will examine the differences in IL-13 secretion and expression between BALB/c mice, C57BL/6 mice and AKR mice. In an attempt to elucidate the effector mechanisms which initiate expulsion, differences in goblet cell numbers between the three strains of mice will be examined as well as differences in the expression of Gob-5, a calcium-activated chloride channel that is thought to be important in increased mucus release.

OP138

**The role of dendritic cells in immunity to Trichuris muris**

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The parasitic intestinal helmhinh *Trichuris trichiura* infects over 800 million people worldwide. Studies using *T. muris*, the murine model of trichuriasis, has provided a number of insights into the immune response to this parasite. Most inbred strains of mice are resistant to infection and expel the parasite. This is dependent on a Type 2 immune response, characterized by IL-4 and IL-13 production. Some strains, however, are susceptible and harbour a chronic infection. This is associated with a Type 1 immune response and IFN-γ and IL-12 production. The C57BL/6 strain used in this study is resistant to infection but expels the parasite at a slow rate. The involvement of dendritic cells (DC) in the immune response to *T. muris* is not well defined. We have begun to characterize the phenotype of bone marrow derived DC exposed to *T. muris* excretory/secretory antigen. We have also shown that mice which receive *T. muris* antigen exposed DC prior to infection show accelerated expulsion of the parasite and increased Type 2 cytokine and parasite specific antibody production compared with control mice. These data support the ability of DC exposed to antigen *in vitro* to induce protective Type 2 immunity to subsequent infection.
Porcine lymphocytes subset(s) responding to recall antigens

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The lymphocyte subsets of the pig are phenotypically unique and complex, but their functions and role in immunity are not well defined. To understand those lymphocyte subsets primed during viral infection and/or after vaccination could define the role of these lymphocyte subsets and may aid in the design of potential vaccines. To address this question, following groups of pigs were prepared: (1) single killed foot-and-mouth disease (FMD) vaccine vaccinated and challenged with FMD virus (2) repeated FMD vaccination without FMD virus challenge (3) DNA based FMD vaccine vaccinated, and (4) African swine fever (ASF) virus infection. Peripheral blood lymphocytes from these pigs were isolated and stimulated in vitro with relevant recall antigens. Proliferation was measured by a H-thymidine (H-TdR) uptake and phenotypes of proliferating lymphocyte subsets were characterized from the same cultures without H-TdR using FACS analysis. Lymphocyte cultures were also prepared for RT-PCR analysis. Almost all of responding cells were CD8+ (except B cells) but the dominant subset(s) of proliferating lymphocytes depends on which type of antigen is used for priming and which recall antigen used for in vitro stimulation.

Development of a single dose mucosal vaccine for anthrax

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Existing licensed anthrax vaccines are administered parenterally and require multiple doses to induce protective immunity. This is not the optimum route for stimulating a mucosal immune response. Microencapsulation of antigens allows intranasal (i.n.) administration of vaccine antigens, stimulating both the mucosal and systemic immune responses with the potential of requiring fewer immunizing doses to achieve protective immunity. We have demonstrated previously that two i.n. doses of microsphere associated recombinant Protective Antigen (rPA), the dominant antigen for protection against anthrax infection, is protective in mice. Due to mouse size restrictions it has not been possible to deliver sufficient microspheres to achieve protection after a single i.n. dose, but preliminary studies have shown that combined administration of microencapsulated rPA with the mucosal adjuvant cholera toxin (CT), is protective following a single i.n. dose. Further studies are being conducted to optimize this as a single dose vaccine formulation and to investigate the potential use of other bacterial proteins as novel vaccine adjuvants. © Crown Copyright 2003 Dstl

Analysis of the role of CD8+ T-cells in bovine tuberculosis in vivo

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Mycobacterium bovis causes bovine TB and can also cause disease in humans. CD8+ T-cells (CD8 cells) have been shown to play a role in immunity to TB. However, no data is available on the role of CD8 cells in bovine TB in vivo. To address this, two groups of calves were infected with M. bovis; after 10 days group A was injected with a CD8 cell-depleting monoclonal antibody (mab) and group B was injected with a control mab for 7 days. In vitro immune responses to PPD-B and PPD-A were measured weekly. After 8 weeks postmortem examinations (PM) were carried out. H TdR incorporation was similar in both groups, but cells from animals in group B showed higher production of specific IFNγ than cells from calves in group A. PM showed that calves in both groups had comparable TB lesions in the lower respiratory tract and associated lymph nodes (LN). However, the extent of lesions in head LN was less in animals in group A. A correlation between the levels of IFNγ and pathology score was found. These experiments indicate that CD8 cells play a role in immunity to M. bovis in cattle by contributing to the IFNγ response. However, CD8 cells may also play a deleterious role by contributing to the immunopathology of bovine TB.

Phenotypic analysis of intraepithelial lymphocytes (IEL) in resistant and susceptible strains of mouse infected with Trichuris muris

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BALB/c mice expel the large intestine-dwelling nematode T. muris between day 21 and 35 post infection (pi), whereas AKR mice fail to expel the worms and harbour a chronic infection. The expulsion of worms by resistant strains of mouse such as BALB/c requires a Th2 type response. However, the local effector mechanism culminating in the expulsion of T. muris is not known. We used flow cytometry and immunohistochemistry to compare the phenotype of large intestinal IEL from AKR and BALB/c mice at various time points pi. The IEL were characterized by their expression of CD3, CD4, CD8α, CD8β, TCRαβ, TCRγδ, B220, CD49b, CD103, CD25, CD30, CD69 and F4/80. There were interesting dynamic changes in the numbers of CD4+ B220−, and CD69+ IEL over the time course of infection, revealing differences between AKR and BALB/c mice. For example, in BALB/c mice the number of CD4+ cells increased, peaking at day 21 pi, then reverted back to naive control levels by day 35 pi. In contrast, the number of CD4− IEL in AKR mice increased and remained elevated throughout the infection. Certain types of IEL may play a key role in the expulsion of T. muris.

Activation of human natural killer cells by Plasmodium falciparum malaria

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The control of acute infection with the human malaria parasite P. falciparum is highly dependent on interferon-γ (IFN-γ). We have shown that natural killer (NK) cells are the initial source of IFN-γ in response to P. falciparum-infected erythrocytes (iRBC). The NK cell response, although stable within an individual, varies between individuals. Here
we show that NK cells form stable conjugates with iRBC, that activation of NK cells depends on direct contact with iRBC and is accompanied by expression of CD94/NKG2A, a member of the C-type lectin family of receptors. It is thus likely that NK activation requires recognition of the parasitized erythrocyte surface by NK cell receptors and it is possible that heterogeneity in the response to iRBC is due to differences in expressed repertoire of activating or inhibitory NK cell receptors. Accordingly, a preliminary genetic analysis has indicated an association between NK responses to iRBC and killer immunoglobulin-like receptor (KIR) genotype. Further work is underway to identify the receptors involved in iRBC recognition and to characterize the genetic determinants of the heterogeneous response.

**OP144**

**Rapid induction of IL-12 from human mononuclear cells by P. falciparum infected red blood cells**

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IL-12 production by human peripheral blood mononuclear cells (PBMC) in response to either lipopolysaccharide (LPS) or Plasmodium falciparum infected red blood cells (pRBC) was investigated in a whole blood assay using FACScan analysis. Both stimuli induced IL-12 in a small proportion of PBMC and were compared in terms of the kinetics of the response and the cellular source of IL-12. P. falciparum induced IL-12 from a single population of cells with the characteristics of activated monocytes whilst LPS induced IL-12 from an additional population with the characteristics of dendritic cells. These data suggest that early cytokine induction during *P. falciparum* infection differs from that of bacterial endotoxin.

**OP145**

**African Swine Fever virus (ASFV) infected macrophage cannot polarize the cytokine/chemokine production profile in vivo**

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ASFV predominantly infects cells of the macrophage lineage and previous studies have demonstrated ASFV infection of macrophage inhibits pro-inflammatory cytokines but enhances production of anti-inflammatory cytokines such as TGFβ. However, it is not clear how the cytokine production profile of ASFV infected macrophages affects cytokine and chemokine production from other lymphocytes *in vivo*. In this study we compared the profile of a wide range of cytokines and chemokines produced by peripheral blood lymphocytes and lymphoid tissues during infection with ASFV isolates of differing virulence by RT-PCR and ELISA at various time points. We also examined the capability of memory lymphocytes to produce cytokines and chemokines upon stimulation with recall antigen *in vitro*. Although the production of IL-1, IL-6 and TNFα was down-regulated by ASFV infection, TGFβ, IL-15 and IFNγ were observed in all ASFV infected animals. IL-8, IL-10 and IL-18 were only observed in animals infected with virulent isolates of ASFV. These data suggest that cytokine production by ASFV infected macrophages does not polarize cytokine and chemokine production in ASFV infected animals.

**OP146**

**The role of CD8+ cells in chronic helminth infection**

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The intestinal murine nematode *Trichuris muris* is not expelled from susceptible AKR mice when given as a high dose infection or when given as a low dose to normally resistant C57BL/6 mice. Both regimes result in chronic infection and a type 1 cytokine response, i.e. high levels of IFN-γ and IL-12. Previous work has highlighted a large population of CD8+ IFN-γ+ cells present within the mesenteric lymph nodes of susceptible animals. In both AKR and C57BL/6 mice the *in vivo* abolition of CD8+ cells either prior to infection or once infection had become established failed to effect chronicity, implying CD8+ T cells are not essential for the initiation or maintenance of the susceptible type 1 response to *T. muris*. Interestingly the percentage of IFN-γ+ CD4+ cells increased in treated groups, perhaps in a compensatory role. The antigen specific cytokine responses were similar in both treated and control groups with the exception of IL-5, which was 4 fold higher in animals receiving anti-CD8 mAbs (YTS168.4). The effects of CD8 depletion and subsequent increased IL-5 production on parasite associated pathology are currently being investigated.

**OP147**

**Mechanisms of eosinophil-mediated killing against Brugia malayi**

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*Brugia malayi* causes the chronic human disease, lymphatic filariasis. Delineating the mechanisms of protective immunity has proved elusive. Utilizing eotaxin and (eotaxin & IL-5) deficient mice, we have previously demonstrated that eosinophils are vital in the killing of microfilariae (Mf) from both tissue (the peritoneal cavity) and bloodstream infection sites. However, the effector mechanism has not been determined. Activated eosinophils are capable of killing many helminth larvae, including several filarial species *in vitro*. Eosinophils may also modulate the immune response through the release of cytokines. Here, we have examined the degranulation status of eosinophils in the bloodstream during infection using an eosinophil peroxidase (EPO) assay. Furthermore, we have isolated an eosinophil enriched population from the peritoneal cavity and studied gene expression using a macroarray. We discuss the direct and immunomodulatory roles of eosinophils in Mf killing.

**OP148**

**Long-term immune control of Cryptococcus neoformans infection by specifically activated human PBMC**

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*Cryptococcus neoformans* is an opportunistic and pathogenic yeast and a significant cause of morbidity and mortality in AIDS patients. This study aims to model *in vitro*, immune control of cryptoccal growth using human PBMC. PBMC were cultured with and without heat-killed *C. neoformans* for 12 days prior to addition of live organisms. The course of infection was followed by CFU counts. Unstimulated PBMC failed to contain *C. neoformans* (strain B3501) growth and by 48 h, macrophages...
full of fungal cells were observed and destruction of the monolayer and extracellular fungal replication followed. In contrast, after pretreatment with killed organisms, the number of viable fungi remained suppressed over at least 10 days. Infection with an avirulent isogenic acapsular isolate (CAP67) was virtually eradicated, whereas strain B3501 broke through immune control after 10–14 days. During immune control, fungal cells were within aggregates of macrophages and lymphocytes. This model is being further developed and the importance of specific cellular components and cytokines tested. The model provides a new tool to investigate mechanisms of human immune control of cryptococcal infection.

### Immunomodulation

**OP149**

**Effect of endogenous intestinal microparticles on the context of antigen recognition**

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The endogenous intestinal microparticle, calcium phosphate (CaPO₄), precipitates in the ileum where it is known to bind bioluminal antigens *in vivo*. Previous work by this group (JP) has demonstrated discrete localization of CaPO₄ to cells in Peyer’s Patches with a morphology and distribution typical of macrophages and dendritic cells. Precipitation of CaPO₄ as *in vivo* in the presence of BM macrophages effects IL-1β production and cell death. When CaPO₄ or 1–1000 ng/mL LPS was added to BM macrophage cultures, there was a distinct difference in both morphology and the production of IL-1β, IL-10 and TGF-β by both groups of cells. Uptake of weakly adherent (apoptotic) CaPO₄ treated cells produced increased levels of TGF-β in culture supernatants when compared to the control. When mice were immunized i.p. with CaPO₄; HEL (hen egg lysozyme) weak recall responses of splenocytes to HEL were observed unlike immunization with CaPO₄ or soluble HEL alone (no response). Therefore these studies indicate that CaPO₄ affects the context of antigen recognition and is thus relevant to the maintenance of intestinal homeostasis and disease both locally (e.g. IBD) or systemic priming (e.g. RA).

**OP150**

**Development of novel reagents to regulate autoimmune disease models**

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Experimental autoimmune encephalomyelitis (EAE) is a disease model for multiple sclerosis (MS), a chronic, inflammatory disease of the central nervous system (CNS), in which invasion by monocytes and T lymphocytes leads to demyelination and axonal injury. Lymphocyte cell surface proteins OX40 and OX2 are involved in immune responses and can be targeted to manipulate autoimmune disease. OX40 belongs to the tumour necrosis factor receptor (TNFR) superfamily and is expressed on T cells. Its ligand OX40L belongs to the TNF superfamily and is found on dendritic cells. OX2 and its receptor belong to the Immunoglobulin superfamily (IgSF). OX2 is widely distributed whereas OX2R expression is restricted to cells of myeloid lineage. In these studies we have targeted the OX40–OX40L and OX2–OX2R interactions in murine models of EAE. The first approach used monoclonal antibodies (mAbs) against OX2R and OX40L. A second involved OX2 recombinant proteins designed to block the OX2–OX2R interaction. These analytical and diagnostic molecular tools were designed to be multivalent to increase avidity. The biochemical characteristics of these reagents, their binding ability and their effect on disease models will be discussed.

**OP151**

**Adjuvant properties of serogroup B meningococcal outer membrane vesicles**

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Meningococcal disease is a worldwide health problem. The causative agent is *Neisseria meningitidis* (Nm) which is an encapsulated, gram negative diplococcus. During the growth of Nm, natural outer membrane vesicles (OMVs) are released from the cell surface containing a high proportion of lipopolysaccharide (LPS) and outer membrane proteins (OMPs) which is representative of the meningococcal outer membrane. Studies have shown NOMVs to be highly immunogenic and to provide a good adjuvant effect on mucosal antibody responses toward respiratory viruses. NOMV components fractionated using preparative SDS-PAGE were assayed for their ability to stimulate cytokine production from U937 cells. Our findings show high levels of TNF-α and IL-10 production by many of the fractions, a few of which showing very high levels of TNF-α but no IL-12 (p70) production. Preliminary results suggest stimulation is not wholly dependent on LPS as similar stimulation is exhibited with a LPS-deficient mutant of Nm.

**OP152**

**The enrichment of CD4⁺CD25⁺ regulatory T cells in the CD4⁺CD45RBbright T cell-subset modifies the functional properties of `memory` T cells**

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Naive and memory T cells are defined by their phenotype, effector function in response to antigens and trafficking patterns *in vivo*. Due to low frequencies of antigen-specific T cells in the alloreactive repertoire of a normal host, these subsets are often characterized using TCR transgenic mouse models. We analysed the phenotype stability and functional properties of these cells selected from nonmanipulated mice. Compared to naive CD4⁺CD45RB⁺CD44⁺ T cells, memory CD4⁺CD45RB⁺CD44⁻ T cells were hyporesponsive to polyclonal or allogeneic stimulation, produced less IL-2 and were prone to early apoptosis after TCR activation *in vitro*. After adoptive transfer in a syngeneic lymphopenic host, both subsets migrated to secondary lymphoid organs and underwent homeostatic proliferation but CD4⁺CD45RB⁺ T cells showed a limited degree of expansion on a cell per cell basis, even after an antigenic challenge. Only the CD4⁺CD45RBbright subset contains CD4⁺CD25⁺ T cells, that when depleted, restored their proliferative capacities. Our findings suggest the
role of regulatory T cells on the homeostasis of memory CD4+ T cells and their response to TCR signals.

**OP153**

**Adjuvant effect of CD40 antibody-antigen conjugates: a direct effect on B cells, and enhanced T cell priming**

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We have shown that CD40 antibodies attached to antigen have a potent adjuvant effect on responses to the antigen. We show here that these conjugates do not by-pass T cell help completely, but do provide a CD40 signal to B cells. The adjuvant effect results however, in increased T cell priming, and passive transfer of the enhanced secondary antibody response is mediated predominantly through T cells.

**OP154**

**Human peripheral blood CD4+ CD25+ T cells regulate Th1 and Th2 clonal responses to aeroallergens**


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Recently a subset of naturally occurring peripheral blood CD4+ T cells has been identified in mice and humans that have the ability to suppress T cell responses in vitro. These CD4+ T cells coexpress the CD25 (IL-2Rα) molecule, they are hyporesponsive and have the ability to suppress CD4+CD25+ T cell responses to polyclonal stimulation in vitro. However, their ability to suppress antigen-specific clonal Th1 and Th2 responses in vitro has yet to be determined. To address this question we generated Th1/Th2 (IFNγ and IL-10) and Th2 (IL-5 and IL-13) clones to cat allergens or peptides. Peripheral blood derived CD4+CD25+ T cells stimulated by the clonal-specific antigens could suppress the proliferative and cytokine response of the autologous CD4+ T cell clones in in vitro mixing assays. The level of suppression was dependent on the ratio of CD4+CD25+ T cells to CD4+ clone (2:1 ratio suppressed proliferation and cytokine production by 50–100%). These experiments provide evidence that human CD4+CD25+ T cells can suppress activation of differentiated Th1/Th0 and Th2 clones. This suggests that such cells may also have the capacity to regulate activated effector T cell responses in vivo.

**OP155**

**A mechanism for expansion of regulatory T cells without loss of function**

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The effect of pathogen products on human CD4+CD25+ regulatory T cell properties is unclear. We have found that regulatory cells constitutively express TLR4, as well as CD25. Pre-incubation with even high doses of LPS or whole, fixed *Escherichia coli* had no effect on suppressive function. Pre-incubation with IL-2 increased suppressive potency. Pre-incubation with bacteria or LPS and IL-2 increased CD4+CD25+ T cell proliferation but with no synergistic increase in suppressive activity. The increased proliferative response after incubation with bacterial products and IL-2 indicates a partial reversal of anergy but without attenuation of regulatory ability. We hypothesize that during bacterial infection, this TLR4 mediated partial reversal of anergy permits sufficient expansion of the regulatory population to maintain an adequate regulatory effector cell ratio.

**OP156**

**The modulation of bovine T-cell responses by glycans of the Mycobacterium bovis antigen MPB83**


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Bovine tuberculosis is a disease of zoonotic and economic importance caused by the intracellular pathogen *Mycobacterium bovis*. Development of vaccines and complementary diagnostic reagents for use in the control of bovine TB will be aided by a greater understanding of factors influencing the recognition of *M. bovis* antigens by the host. In the current study, we have investigated the influence of protein glycosylation on the recognition of the native *M. bovis* glyco-protein MPB83 by bovine T-cells. The chemical removal of glycans from a native form of MPB83 resulted in an improvement in its recognition by T-cells from *M. bovis* infected cattle. Further studies suggest that the modulation of T-cell responses by these glycans may be attributed to their influence on antigen processing rather than differential recognition by different T-cell subsets.

**Inflammation**

**OP157**

**CD4+ CD25+ regulatory T cells suppress the differentiation and functions of Th1 and Th2 cells: Leishmania major infection and colitis in mice**

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Regulatory T cells are critical in regulating the immune response and therefore play an important role in the defence against infection and controlling autoimmune diseases. Here we provide direct in vitro and in vivo evidence that CD4+CD25+ regulatory T cells inhibit the differentiation and functions of both Th1 and Th2 cells. Importantly, CD4+CD25+ T cells suppress the disease development of *Leishmania major* infection in SCID mice reconstituted with naive CD4+CD25- T cells at an early stage in the infection. Furthermore, CD4+CD25+ regulatory T cells not only suppress the induction and functions of both Th1 and Th2 cells in vitro, and also they inhibit the development of colitis induced by both Th1 and Th2 cells in SCID mice. In summary, we demonstrate here that CD4+CD25+ regulatory T cells effectively suppress the induction and functions of Th1 and Th2 cells crucial to the outcome of infectious and inflammatory diseases.

**OP158**

Withdrawn

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OP159
Bystander-activated lymphocytes in inflammation: a natural process?
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The potential role of bystander-activated lymphocyte effector function in inflammation has previously been described. Data shown here characterizes these lymphocytes in terms of their proliferation and apoptosis kinetics, phenotype and cytokine profile. Lymphocytes activated with either IL-2/IL-6/TNFζ or IL-15 proliferated over 8 days after an initial lag phase of 2–3 days. From day 8 a reduction in proliferation was seen with a corresponding increase in apoptosis from day 5. After 8 days culture with cytokines whilst the proportion of T cells did not alter, that of NK cells increased by up to 3-fold with the expression of activation markers increasing on both cell types. The cytokine profile of these lymphocytes was measured and GMCSF, LTα and IFNγ production was shown to increase over 8 days. However IL-10 or TGFβ was not detected. In contrast lymphocytes activated for 48 h with cross-linked anti-CD3 did produce IL-10 and TGFβ in addition to IFNγ, GM-CSF and LTα. The factors mediating bystander-activated lymphocyte effector function are not fully understood but characterization of these lymphocytes may help explain the abundance of TNFζ seen in some inflammatory diseases.

OP160
Regulation of haeme oxygenase-1 expression in mononuclear cells by interleukin-10 and lipopolysaccharide
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Interleukin-10 (IL-10), is a potent anti-inflammatory cytokine, which has recently been shown to induce the expression of heame oxygenase 1 (HO-1). This stress response protein is intimated to have anti-inflammatory and cyto-protective roles within numerous cell-lines and animal models. Herein, we show that although in the RAW264.7 cell-line HO-1 expression is induced by IL-10, LPS can also stimulate the production of this enzyme. In primary human monocytes and in vitro differentiated macrophages, HO-1 is induced by IL-10 via STAT-3 and PI3-kinase signaling however, in these cells LPS inhibits expression of HO-1. Additionally we show by the use of the HO inhibitor Zinc-(II)-Protoporphyrin-IX, IL-10 suppression of LPS induced cytokine production is independent of HO-1 activity.

OP161
Serum amyloid P interaction with FcγRIII
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Investigations on the interactions between pentraxins and Fc receptors, derived from E. coli, were performed using surface plasmon resonance. No interactions could be seen between the soluble extracellular domains of FcγRIIA, FcγRIIB or FcγRIII and human recombinant or purified C-reactive protein. Interactions were, however, observed between CRP’s homologue; Serum Amyloid P component (SAP), and FcγRIII with a dissociation constant of approximately 2.6 x 10^-9. Weak binding was observed to FcγRIIB and weaker still to FcγRII. As previously observed for CRP binding to FcγRI, the interactions between SAP and FcγRIII were calcium dependent. FcγRIII is a major Fc receptor present on neutrophils. We investigated the ability of SAP to alter responses to neutrophil activation through FcγRIII. We were able to observe that the incubation of neutrophils with SAP (1–50μg/mL) caused a down regulation of intracellular reactive oxygen production responses measured with dihydroorhodamine. This occurred in isolated neutrophils or in neutrophils stimulated with immune complex. Results will also be presented for other neutrophil receptors and other stimuli.

OP162
An investigation of human serum amyloid A protein interaction with microorganisms
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Triggered by inflammation or stress, serum amyloid A (SAA), increases up to a 1000-fold above physiological levels. The rise in SAA levels is induced by inflammatory cytokines such as TNF-α, IL-1β and IL-6. SAA has been reported to bind to a variety of cellular ligands including extracellular matrix glycoproteins, lipoproteins such as HDL and receptors, RAGE and fPRL1. For the first time we show that SAA binds to a range of bacteria including Escherichia coli and Pseudomonas aerugi-nosa. Binding of SAA to bacteria was shown with flow cytometry and phthalate oil experiments. Ligan blotting experiments have identified the bacterial ligand at a molecular weight of 35 and 29 kDa. Escherichia coli BL21 has shown to bind SAA via 35, 29 and 14 kDa. Isoelectric focusing demonstrated a PI of 4.6–6 for the ligand. Western blot of partially purified fractions of E. coli allowed identification of the ligand using MALDI-TOF MS analysis of peptide sequences. SAA’s bacterial ligand of 35 and 29 kDa is outer membrane protein A and the 14 kDa ligand is outer membrane X found in gram negative bacteria.

OP163
Innate immune functions of serum amyloid A
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Acute-phase Serum Amyloid A (A-SAA) are amongst the major acute-phase proteins whose in vivo plasma concentrations can increase by as much as 1000-fold during inflammation. There have been conflicting findings relating to the functions of A-SAA such as the oxidative burst response, chemotaxis and degranulation. Recently, we identified an ability of A-SAA to bind to a variety of bacteria. In this study, A-SAA was successfully purified from human plasma and its binding to Escherichia coli via 35, 29 and 14 kDa ligand/s was shown by phthalate oil and ligand

innate immunity

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blotting experiments. It was demonstrated that SAA tends to aggregate which induced more TNFα and IL-8 production by macrophages and neutrophils, respectively, compared to the monomeric SAA. Both monomeric and aggregated A-SAA enhanced binding and phagocytosis of E.coli by neutrophils and macrophages. These results suggest a role of SAA in host defence and hence in innate immunity.

OP164  
CpG DNA protects against the live vaccine strain (LVS) of Francisella tularensis but not the fully virulent strain HN63  
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Stimulation of innate immunity by CpG DNA (via TLR9) has been shown to protect against a number of intracellular pathogens. Elkins et al. demonstrated that a single dose of CpG DNA administered 3 days before challenge offers protection to 10^7 cfu F. tularensis LVS i.p. in the BALB/c mouse model. However, whilst LVS is virulent in mice it is attenuated in man. Therefore, CpG mediated protection to the fully virulent holarctic strain HN63 was tested. Whilst complete protection was achieved to LVS by the CpG ODN A2, no protective effects were observed to HN63 administered intraperitoneally or subcutaneously. Sera collected on the day before challenge was analysed by cytokine expression. Animals treated with CpG DNA were found to have increased levels of the inflammatory cytokines IFN-γ and TNF-α compared to controls. In vitro models of F.tularensis infection have been developed in monocye/ macrophage cell lines. Stimulation of these cells with CpG DNA does not result in a reduction in the intracellular growth of LVS, suggesting that the CpG DNA mediated protection achieved in the mouse model is not at the macrophage level.  
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OP166  
Withdrawn

OP165  
Bioinformatic discovery and initial characterization of nine novel antimicrobial peptides in the chicken  
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Antimicrobial peptides (AMPs) are an essential component of innate immunity in a range of species from Drosophila to humans and are generally thought to act by disrupting the membrane integrity of microbes. In an age when antibiotic resistance is an increasing problem, these peptides are of interest as novel pharmaceutical agents. Here we describe the discovery of nine novel AMPs in the chicken, an economically important agricultural and model organism. We have implemented a bioinformatic approach that involves the clustering of more than 420,000 chicken Expressed Sequence Tags (ESTs). Similarity searching of proteins, predicted to be encoded by these EST clusters, has resulted in the in silico identification of full-length sequences for seven novel gallinacins (Gal-4 to Gal-10), a novel cathelicidin and a novel liver expressed antimicrobial peptide 2 (LEAP-2) in the chicken. We have performed an evolutionary analysis of the gallinacin family and detected sites that are under positive selection in these molecules. We have also characterized the expression levels of LEAP-2 mRNA in a panel of chicken tissues post infection with a caecal parasite (Eimeria tenella) and the expression of the novel gallinacins in a chicken liver cell line following stimulation with either LPS or Eimeria sporozoites.

OP167  
CD8α expression identifies a CD56~/3-NK cell subset with discrete function  
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We have previously shown that CD8^+ NK cells are more cytotoxic than their CD8^- counterparts and are involved in antileukaemia activity in vivo; their activity being correlated with continued remission. Here we have investigated differences between the CD8^+ and CD8^- NK subsets with respect to NKIR, natural cytotoxicity receptor (NCR) and adhesion molecule expression, response to cytokines and effects of target cell conjugation. CD8^+ cells showed no differences in NKIR expression but NCRs were more frequently expressed and at higher densities. Culture in IL-2/15 enhanced these differences. No differences were apparent in adhesion molecule expression on resting NK cells although culture in IL-2/15 led to higher expression of CD11a and CD54 on the CD8^- cells. Strikingly, conjugation of CD8^- NK cells to K562 target cells induced apoptosis (annexin V expression) within 24 h which was not the case in the CD8^- subset. This increased susceptibility to apoptosis in CD8^- cells was not overcome by the addition IL-2 or IL-15. This is the first report of AICD in NK cells. The role of the CD8 molecule in these findings remains unclear; ligation with mAb, although activatory, did not induce any of these changes.  

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Macrophages

OP168
Investigation of disease pathogenesis in a model of infection-induced reactive arthritis
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Reactive Arthritis (ReA) is a sterile joint inflammation triggered by genitourinary or gastrointestinal bacterial infection (e.g. Salmonella typhimurium (ST)) and is strongly associated with HLA-B27. Bacterial antigen has been detected in synovium from ReA patients, but how it is transported there is unknown. Macrophages (Mφ) could be responsible and may also have a role in antigen presentation. We test this hypothesis based on a model of oral C5TS (temperature sensitive ST) infection induced ReA, by characterization of Mφ activation after ST infection in vitro and adoptive transfer of infected Mφ into HLA-B27 transgenic (B27) mice. In vitro infection with C5TS of bone marrow derived Mφ from B27 mice up-regulates CD40 and CD54 and induces IL-6, IL-12, TNF-α and nitric oxide in a manner similar to LPS. Mφ infected with recombinant ST expressing a CTL epitope stimulate the specific CTL. Preliminary data, after adoptive transfer to B27 mice, shows that C5TS infected Mφ are detected in the Peyer’s patches and mesenteric lymph nodes in greater numbers than LPS or uninfected Mφ. We are currently testing whether ReA can be reconstituted by ST infected Mφ transfer with or without T cells in B27 mice.

OP169
Microarray analysis of the murine macrophage response to LPS and CpG
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Macrophages play a crucial role in the innate immune response to bacterial pathogens and become activated following stimulation by bacterial components known as pathogen associated molecular patterns (PAMPs) e.g. LPS and bacterial DNA. PAMPs cause macrophage activation by binding to pattern recognition receptors on the cell surface, initiating gene transcription and causing metabolic and biochemical alterations within the cell. Genes for cytokines, chemokines and other inflammatory mediators are expressed and these molecules are released by the cell propagating the immune response. A time-course study was performed on the murine macrophage cell line J774.A in order to determine gene expression patterns following activation with LPS (Salmonella typhimurium) and bacterial DNA (CpG ODN1826). Genes expressed in response to macrophage stimulation were studied using a custom designed oligonucleotide microarray. The microarray encompassed over 1000 genes of interest from a variety of cellular processes including cytokines and chemokines known to be expressed during infection. The results presented here provide a detailed examination of changes in gene expression in macrophages in response to PAMPs.

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OP170
CGR7 signalling regulates localization of marginal zone macrophages
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The marginal zone (MZ) of the spleen is known as an important site for capturing blood-borne pathogens. We previously reported that visceral leishmaniasis is associated with changes to the structure of MZ, notably a loss of MZ macrophages (MZMs), may cause immunosuppression. As a basis for this phenomenon during chronic infection, we have investigated how MZMs maintain their anatomical distribution in normal, uninfected mice. Here we report that splenic mice, which lack CCL19 and CCL21, have far fewer MZMs compared with normal C56BL/6 mice. Moreover, administration of Pertussis toxin (PTX), an inhibitor of chemokine receptor signalling, to C56BL/6 mice result in MZMs exiting MZ and becoming localized in the red pulp. This effect of PTX is transient, with MZMs subsequently reappearing in MZ. MZMs can also migrate in vitro in response to CCL21 as well as CCL19. Collectively these data suggest that CCR7-mediated responses to these chemokines may play a role in the localization of MZMs in the spleen. The stromal cell source of CCL21 in the spleen is dramatically reduced during chronic infection (Ato et al. Nat. Immunol. 2002 3 : 1185) thus provides one possible explanation for the loss of MZMs seen during this disease.

OP171
Pro-inflammatory macrophages: partial differentiation or preferential pathway?
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Macrophages are a major cytokine source in inflammatory diseases such as RA. Cytokine imbalance suggest selective activation or selection of pro-inflammatory macrophages. Activation determines monocyte cytokine profile but has little influence on macrophages: differentiation is more influential, the factors used argue against selection of pro-inflammatory cells. M-CSF drives monocyte differentiation resulting in a pro-inflammatory intermediate which upon further differentiation becomes anti-inflammatory. Cytokines or apoptosis may block progression to anti-inflammatory macrophages. Results show that although M-CSF macrophages are insensitive to Fas, they are sensitive to apoptosis induced by TNF and aged monocytes. In addition, maturation was blocked by RA supernatants. Description of conventional and alternatively activated or M1/M2 differentiated macrophages lead us to investigate whether a preferential differentiation pathway exists in chronic inflammation; M1 were predominantly pro-inflammatory and M2 or M-CSF cells were discriminatory for IL-10 production. In conclusion a subtle balance between apoptosis, differentiation and activation maintain tissue macrophages in a pro-inflammatory state.
Mucosal immunology

OP172
Intranasal delivery of E. coli heat-labile toxin B subunits (EtxB) prevents autoimmune diabetes mellitus in NOD mice
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Non-obese diabetic (NOD) mice spontaneously develop diabetes caused by autoimmune T cell-mediated destruction of pancreatic β cells. EtxB, a structural homologue of CtxB, is a highly stable nontoxic and an immunomodulatory agent capable of abrogating T cell mediated autoimmune disease. Intranasal treatment of female NOD mice with 10 μg EtxB on 8 occasions on alternate days dramatically reduced the incidence of diabetes. Disease protection is associated with a significant reduction in the number of macrophages, CD4+ T cells, B cells, MHC class II + cells infiltrating the pancreatic islets. Despite this, treated mice showed increase number of IL-10 + cells in the pancreas and there was a decrease in both Th1 and Th2 cytokines production in the pancreatic lymph node. Disease protection could also be transferred with CD4+ splenocytes from treated mice and was associated with a similar histopathologic and cytokine-secreting profile. The implication of the result to the development of treatment regime in diabetes disease is discussed.

OP173
Gender related differences in the gastric mucosal response to long-term chronic Helicobacter pylori infection in the Mongolian gerbil
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The Mongolian gerbil (MG) is a good model for long-term chronic H. pylori infection leading to cancer. This study examined gender differences in the gastric cytokine responses to H. pylori. Infected (H. pylori SS1 strain) MGs and age/match-controled mice were sacrificed at 36 weeks. Gastric mucosa was taken for histology and analysis of cytokine transcripts by RT-PCR. H. pylori infected females, but not males, had increased (P < 0.01) gastric IFNγ transcripts compared to controls. IFNγ levels in infected males were lower (P < 0.05) than in females. IL-12p40 transcripts were lower in males, infected (P < 0.05) and controls (P < 0.05), than females. However, IL-12p40 was increased (P < 0.01) with infection in both genders compared to controls. In H. pylori infected MGs there was a correlation (P = 0.001, r = 0.648) between IFNγ and IL-12p40 transcript levels. IFNγ, but not IL-12p40, correlated with chronic (P < 0.001, r = 0.673) and active (P < 0.05, r = 0.463) inflammation scores in the antrum. H. pylori stimulates a Th1 cytokine response in MGs, with IFNγ correlating with inflammation. Gender differences in cytokine responses have been observed.

OP174
Adult stem cells in the human intestine
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Stem cells resident in the small intestinal crypts constantly give rise to epithelial cells of the villi. The small intestine is also recognized as a site of lymphoid development in mice. It is possible that haematopoetic stem cells (HSCs) are present in adult human gut, giving rise to lymphoid cells. Detection of recombinate activating gene (RAG) transcripts and IL-7 and IL-15 in the human intestine support this hypothesis. In this study, multiparameter flow cytometry was used to detect and characterize haematopoetic stem cells (CD34+ CD45−) in the epithelium (EL) and lamina prapra (LP) of human small intestine. Biopsies and blood were taken from nondiseased patients (n = 12) and patients with coeliac disease (n = 8). HSCs were detected in the EL and LP of all samples at levels significantly higher than peripheral blood (P < 0.05). There were significantly more HSCs in the LP vs. the EL of normal patients (P < 0.05). Levels of HSCs in the EL of the coeliac patients were significantly lower than that in normal patients (P = 0.02). This is the first demonstration of HSC’s in human small intestine, which, along with the RAG, IL-7 and IL-15 data, supports the hypothesis that lymphoid cells are developing locally.

OP175
Effect of feeding n-3 polyunsaturated fatty acids on mouse colitis
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The n-6 and n-3 polyunsaturated fatty acids (PUFAs) generate different eicosanoids by cycloxygenase and lipoygenase pathways. Products of n-3 PUFAs are anti-inflammatory, whereas n-6 PUFAs are pro-inflammatory. In human trials, n-3 PUFA reduced the severity of colitis. We examined the effect of feeding n-3 PUFAs on colitis induced in SCID mice by transfer of CD45 Rhb+ T cells. Mice fed on a diet enriched for n-3 PUFAs gained weight, whereas control mice lost weight. Transplanted control-diet mice had colitis and high pathology scores (range 5–8, mean 7.25). Mice receiving n-3 PUFAs had no macroscopic colitis and lower pathology scores (range 3–8, mean 4.6). Mice on n-3 diet had more mucosal dendritic and CD11b+ cells, but fewer PMN, monocytes and macrophages compared to control mouse. T cell infiltration was similar in both groups. Control colitic mice had elevated levels of colonic IL-1β, TNFα and IL-12 compared to n-3 PUFAs fed mice, but no difference in IL-10 levels. There was increased submucosal collagen in the n-3 group, but mucosal myofibroblasts were more activated in the controls. Results indicate n-3 PUFAs modulate mucosal immune cell activity, rather than cell recruitment.
OP176
Essential role for CD103 in the T cell-mediated regulation of experimental colitis

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The T cell-mediated regulation of potentially pathogenic immune responses is a well-established but incompletely understood phenomenon. CD103 (αE-integrin) is one of the molecules proposed to identify subsets of regulatory CD4+ T cells, but data about its function are scarce. We therefore addressed the role of this molecule in immune regulation by using the T cell transfer model of colitis. CD4+CD25+ T cells from CD103−/− donors could prevent CD4+CD45RBhigh T cell-induced colitis in scid recipients, showing that CD103 is not mandatory for the function of regulatory T cells. In contrast, wildtype (wt) CD4+CD25+ T cells were unable to protect CD103−/− Rag−/− recipients from T cell-induced colitis, demonstrating that intestinal immune regulation required host cells to express CD103. Further investigation showed that CD11chigh cells contain a sizable proportion of CD103− cells. These large-sized cells, present also in wt animals, were B220−, Gr-1−, CD11b+ and MHCII+high. While the majority of these cells were CD8α+ in the spleen, they were CD8α+ (as well as CD134L−) in the mesenteric lymph nodes. The mechanism by which these cells are involved in the regulation of colitis is currently under investigation.

OP177
Characterization of a novel model of intestinal inflammation

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The mucosal immune system must be tightly regulated in order to remain responsive to pathogenic antigens whilst remaining unresponsive to ubiquitous antigens such as the normal flora and food proteins. Models whereby this regulation has been abrogated provide powerful tools for the study of mucosal as well as peripheral tolerance. Most models of gut inflammation require manipulation of the host, either via transfer of activated cells or via chemical means. Very few models of spontaneous disease, are available, particularly in the rat. Here we report the characterization of a novel model of spontaneous gut inflammation, the Lymphopenic (Lyp/Lyp) PVG rat, which has been derived from the crossing of the lymphopenic gene from the diabetic BB rat on to the PVG R11u background. This rat displays a chronic inflammation involving both small and large intestine, the median onset of which is around six months of age. Data will be presented showing this model to be characterized by a striking eosinophilic infiltrate, a high serum level of IgE and IL-4 T cell production, overall suggesting a Th2 type disease phenotype.

T cells

OP178
Production of FOXP3+CD4+CD25+ T regulatory cells: a role for cortical epithilium in central tolerance

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Mechanisms of T cell tolerance involve intrathymic negative selection and the production of CD4+CD25+ T cells with regulatory ability (Treg). It is well established that thymic dendritic cells and medullary epithelium mediate negative selection. In contrast, the mechanisms regulating intrathymic T reg production are unclear. Moreover, the relative contributions that negative selection and T reg acts actually make to tolerance are poorly defined. Using in vitro and in vivo approaches we have analysed the developmental requirements for Tregs in the thymus and investigated their involvement in acquiring T cell tolerance. We show that Tregs are first detected as CD4+CD8− thymocytes that have initiated FOXP3 expression during positive selection, as a result of interactions with cortical epithelium. Furthermore, we show that Tregs alone are insufficient at maintaining tolerance to self. Therefore T cell tolerance reflects a balance between positive selection of self specific thymocytes by cortical epithelium, negative selection of autoreactive specificities by medullary epithelium and thymic dendritic cells, and T reg production by cortical epithelium.

OP179
Long-term culture of NKT cells results in a decreased cell proliferation and CD28 down-regulation

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NKT cells represent a novel T cell lineage characterized in humans by a TCR with an α chain encoded by Vα24-JαQ, paired preferentially with a Vβ11 chain. Evidence from studies using aged T lymphocytes, as well as T lymphocytes after long-term invitro cultures have shown that some of the alterations associated with T cell immunosenescence include a decreased proliferation and IL-2, decrease of CD28 expression. We have studied the proliferative capacity and the expression of CD28 and NK cell markers, CD161, CD94 and CD85 in NKT cells in long-term invitro culture. In these cultures, PBMCs from healthy young donors were stimulated repeatedly with α-galactosylceramide and IL-2. Our results demonstrated that α-galactosylceramide induced the proliferation of NKT cells although the expansion of NKT cells in response to α-galcer decreased after second week of culture. The expression of CD28 on NKT cells also decreased during culture, whereas the expression of NK cell markers did not change significantly. These results suggest that NKT cells aged invitro by repeated specific-antigen stimulation present alterations compatible with the process of T lymphocyte immunosenescence.
OP180
Functional characterization of HLA-A68 restricted cytotoxic T cell responses
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The class I MHC molecule HLA-A68 contains a substitution at position 245 (Ala > Val) that impairs binding of the T-cell coreceptor CD8. To investigate the implications of this for HLA-A68 restricted T-cell responses, tetrameric forms of HLA-A68 protein were produced, refolded around immunodominant cytomegalovirus or influenza peptides. Significant in vivo CTL responses were observed against HLA-A68 restricted epitopes. The phenotype of such cells was typical of virus-specific immune responses against other HLA alleles. TCR Vβ usage was highly focussed on individual Vβ segments within individuals, with little sharing of TCRVβ segments between individuals. HLA-A68 restricted T cell clones were established and only a minority (2/7) were functionally CD8 independent. Functional avidity and TCR affinity of the clones were tested in peptide titration and HLA-peptide tetramer dissociation assays, respectively; no significant differences were universally observed compared to control clones. In summary, functional CD8+ T cell immune responses are directed against HLA-A68 restricted peptides, despite weak CD8 binding to this allele. The biophysical properties of the underlying TCR/MHC interactions are being studied.

OP181
Telomere length in the different T cell subsets from healthy elderly donors
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We have previously studied that the T cells show a decrease in the expression of CD28 in the elderly, associated with an increase of the expression of some NK-R in the CD28 negative subsets. The aim of this work is to analyse the telomere length in the different lymphocyte subsets from PBMCs from healthy elderly donors compared with young controls. Our results show that the CD28- subset present a shortening in the telomere length when compared with the CD28+ subset both in young and elderly. This result is completed by the finding that there is a decrease in the telomere length associated to ageing in the CD28+, CD28- and NK cell subsets. These results support the hypothesis that these cells are cytotoxic T cells that has undergone a process of replicative senescence. Thus we can confirm that the telomere length is not only a good biomarker of ageing; there is also a biomarker of replicative history probably associated to chronic activation.

OP182
Studies of B7-1 and 4-1BBL costimulation in human T cell activation
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Co-stimulation of T cells through a variety of ligand–receptor interactions plays an important role in shaping the immune response to engagement of the TCR-complex. A better understanding, and the ability to manipulate these processes may help to promote beneficial antitumour immune responses in cancer patients. As a model system, we have used A549 lung carcinoma cells infected with replication-defective adenovirus vectors expressing B7-1 (CD80) and/or 4-1BBL (CD137 ligand), to deliver these costimulatory signals to cocultured PBMC, in the presence of anti-CD3 mAbs. Proliferation of T cells was monitored by 3H-thymidine incorporation, and by CFSE labelling in conjunction with flow cytometry. Initial data confirm that during the early days of culture, B7-1 (±4-1BBL) supports anti-CD3 stimulated T cell proliferation in a dose-dependent manner, while 4-1BBL alone is less effective. After 7 and 14 days, the combination of B7-1 and 4-1BBL with anti-CD3 sustains lymphocyte proliferation significantly better than either costimulator alone. Immuno-phenotyping of the cultures shows more cells of a mature (CD45RO+, CD62L−) and activated (CD25+) phenotype resulting from double costimulation.

OP183
Genetic influences on antigen specific human regulatory T cell responses
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Our previous characterization of CD4+ T cell responsiveness to a major blood group antigen, the RhD protein, provides an opportunity to analyse genetic influences on immune regulation. D-negative individuals differ in their ability to produce anti-D antibody, and their CD4+ T cell response also varies. High antibody levels are associated with strong proliferative responses to multiple RhD epitopes, whilst IL-10 and TGF-β responses are prominent in low responders. We typed our panel of immunized donors for single-nucleotide polymorphisms in promoter regions that have been shown to influence cytokine production, including those for IL-10 (−1082 G/A, −819 C/T, −592 C/A) and TGF-β (−509 T/C). These polymorphisms are not significantly correlated with serum antibody levels, or proliferative or cytotoxic responses. In contrast, responsiveness is strongly associated with HLA-DR type: DRB1*15 donors have higher antibody levels and weaker IL-10 responses to significantly fewer epitopes than donors with other types. Our interpretation of these results is that the response to the RBD protein is controlled by specific T-regulatory cells, whose activity is influenced more by class II type than cytokine gene polymorphisms.

OP184
The nature of CD8+ T cell memory to Mycobacterium bovis
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Little information is available on the development of CD8+ T cell memory to chronic bacterial infections. Bovine tuberculosis is a chronic infection caused by M. bovis, that provides a utilisable model for the study of CD8+ T cell memory. Flow cytometric analysis of peripheral blood cells showed that the percentage of CD8+CD26− and CD8+CD45RO+ T cells increased with age, and that most CD8+CD26− T cells were also CD45RO+. A similar percentage of FACS sorted CD8+CD26− and CD8+CD26+ T cells produced IFN-γ after mitogenic stimulation. A reduction in the level of CD62L expression has been proposed as a marker of antigen primed T cells. In agreement with this, FACS sorted CD8+CD62L− T cells had greater capacity than CD8+CD62L+ T cells to produce IFN-γ after mitogen stimulation. Analysis of recall responses from M. bovis–BCG vaccinated cattle boosted with modified vaccinia Ankara virus expressing mycobacterial Ag85, showed that the
CD8⁺ and CD8⁻ CD62L⁻ T cell subsets proliferated and produced IFN-γ upon restimulation in vitro with mycobacterial antigens while the reciprocal populations did not. The results of this study suggest that CD8⁺ T cell memory in cattle are likely to be found in the CD26⁻ and CD62L⁻ subsets.

OP185
Fas-mediated death in avidity-based regulation of autoreactive T cell function

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T cell avidity for self antigen can adapt (‘tune’) via different mechanisms. Biochemical desensitization has been observed in vitro in transgenic T cells cultured with a superagonist analog peptide. In contrast, we have previously shown that in vivo exposure to superagonist peptides based on the encephalitogenic Ac1–9 peptide of myelin basic protein (MBP) led to deletion of the high affinity cells. Thus EAE did not develop. To further assess the mechanism of superagonist-induced tuning in our model, we established an in vitro system to measure T cell death in response to antigenic stimulation. Simultaneously, we have used MBP (Ac1–9)-specific TCR transgenic T cells to investigate whether biochemical tuning to superagonist occurs when TCR affinity is uniform. Our results indicate that evidence for biochemical tuning is marginal at best while T cell death is mediated largely by T cell-to-T cell Fas/FasL interactions and antigen dose. Results were confirmed in vivo in EAE experiments.

OP 186
Isolation of lipid rafts from Jurkat T lymphocytes by flotation through a continuous density gradient

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Recent data indicate that the plasma membrane of mammalian cells is not a uniform phospholipid bilayer. Rather, it is heterogeneous and contains domains enriched in cholesterol and sphingolipid, known as lipid rafts. These domains are able to recruit or exclude membrane proteins and are important in many cellular functions such as signal transduction and cell migration. Rafts are insoluble in detergent solutions including Triton X-100, may be labelled with cholera toxin B subunit (CTxB), a ligand for raft component ganglioside GM1, and can be purified by flotation in density gradients. In this study, rafts were isolated from lysates of Jurkat T cells by ultracentrifugation through continuous gradients of Optiprep™. Before lysis, rafts were labelled with CTxB conjugated to horseradish peroxidase. After ultracentrifugation at 250,000 g for 2.5 h, gradient fractions were collected. Fractions with high peroxidase activity were identified with ABTS substrate. Refractive index measurements indicated rafts accumulate at densities of 1.03–1.08 g/mL. In further experiments proteins from fractions containing rafts or soluble membrane were analysed.

OP187
The influence of coreceptor palmitoylation and raft association on thymic selection

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During thymic selection, negative selection is associated with strong TCR signalling. Signalling intensity is dependent upon the differential expression of coreceptors, including CD8. CD8αβ expressed by conventional T cells combines with the TCR to recognize peptide antigens presented by MHC class I. The CD8β chain contains a palmitoylation signal that allows the CD8 heterodimer to enter lipid rafts. These microdomains are enriched in signalling molecules that may enhance signal strength following TCR engagement. If the palmitoylation site of CD8β were mutated, a conventional T cell that recognizes autoantigen with a high affinity may inappropriately evade negative selection due to a reduction in levels of signalling emanating from the TCR. To test the hypothesis that lipid raft localization is required for optimal signalling associated with negative selection, CD8β mutating the palmitoylation site have been generated. These will be introduced via retroviral transduction into immature thymocytes derived from a CD8β⁻/⁻ mouse and their ability to reconstitute the CD8 compartment characterized using RTOC. Negative selection will be tested using thymocytes derived from a HY TCR transgenic mouse.

OP188
Recirculating CD4 memory T cells are key inducers of secondary antibody responses

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Subcutaneous immunization primes naïve CD4 T cells in the draining lymph nodes, leading to differentiation of CD4 effector T cells that interact with B cells in the T zone, or colonize follicles of the responding node where they select germinal centre B cells. Other effector leave the node and may be recruited to sites of inflammation. In addition to these three types of CD4 effectors, recirculating memory T cells are produced that migrate through the T zones of all peripheral nodes. We report that recirculating memory CD4 T cells are capable of making accelerated secondary responses without the contribution of residual effectors. This is deduced from the rate of secondary responses in nodes involved in the primary response being no faster than that in remote lymph nodes that had not been previously exposed to antigen. Both sets of nodes react considerably faster than naïve lymph nodes. This effect is neither attributable to the number of antigen responsive T cells nor antigen-presentation by B cells. Finally, it is shown that recirculating memory cells are formed without a prolonged effector phase, as these appear in nonresponding peripheral lymph nodes by 3 days after immunization.

OP189
Murine CD4⁻ CD25⁻ Tregs: towards the identification of molecular markers

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Despite intense interest in CD4⁺CD25⁻ regulatory T cells (Tregs), little is currently known about their mechanisms of action and the surface molecules important in suppression. Our aims were therefore: (i) to compare the proteomes of freshly isolated and anti-CD3/CD28 bead-activated CD25⁻ Tregs with equally treated CD25⁺ T cells, using 2-D gel electrophoresis; and (ii) to identify sFv antibodies binding specifically to CD25⁻ Tregs (‘positive’ sFv) using phage display technology. Analytical 2-D gels were prepared from lysates of 5 × 10⁶ BALB/c CD25⁻/⁻ T cells. Comparing gels of CD25⁻ T cells with those of CD25⁺, 157 spots were unique to CD25⁻ and 40 were ≥2-fold more intense; comparing gels of
activated ('') CD25⁺ T cells with those of 'CD25⁻', 83 spots were unique to 'CD25⁻' and 26 were ≥2-fold more intense. After 5 rounds of selection of phage-sFv (Tomlinson Library I) against freshly isolated CD25⁺ Tregs, 8 genetically distinct, positive clones were identified, of 24 randomly screened so far. Ongoing work focuses on the identification of both the differentially regulated proteins by mass spectrometry, by mass spectrometry, and the targets of the positive sFv by immunoblotting 1-D gels of CD25⁺ membrane preparations.

OP190

The interrelatedness and independence of TCRγδ⁺ and TCRζδ⁺ cells

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Whereas T cell receptor (TCR) γδ⁺ and TCRζδ⁺ cells are commonly regarded as functionally independent, few experiments have explored their relatedness and potential interdependence. This study identifies a gene profile distinguishing γδ cells from conventional ζδ T cells. However, rather than being TCRγδ-specific, this profile is shared by unconventional ζδ T cells. Monitoring the profile revealed an abnormality in γδ cell development and function in TCRζδ⁻ mice that mapped to the absence of late-stage ζδ progenitors. Thus, rather than being a unique link between innate and adaptive immunity, γδ cells are better viewed as prototypes of unconventional T cells expressing either TCRγδ or TCRζδ, the development and function of which is strongly influenced by conventional ζδ T cell development.

OP191

Expression of CD154 by Th cells is essential for protective Th1 responses to irradiated Schistosoma mansoni

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The role of the CD40-CD154 (CD40L) signalling pathway was examined in the development of protective Th1 responses induced by the radiation-attenuated schistosome vaccine. Expression of CD154 in the skin-draining lymph nodes increased on both CD4⁺ and CD4⁻ cells following vaccination, although paradoxically expression of CD154 mRNA fell. Vaccination of CD154⁻/⁻ mice indicated that CD154 expression by CD4⁺ cells is essential for successful Th1 responses in the sdLN. Expression of CD154 is also essential for efficient antibody responses. Ligation of CD40 using an agonistic αCD40 mAb completely restored IFNγ expression in the skin- and lung-draining lymph nodes of CD154⁻/⁻ mice, although antibody responses remained deficient. Moreover, treatment of wildtype mice with αCD40 mAb boosted the strength of the established Th1 response. This increased Th1 polarization requires both IL-12 dependent and IL-12 independent pathways. The effect of αCD40 mAb treatment on vaccine-induced protection in wildtype and CD154⁻/⁻ mice will also be described.

OP192

CD8 null pMHC tetramers enable an assessment of CTL quality

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Soluble pMHC multimeric complexes have proved invaluable tools for the identification and characterization of antigen specific Cytotoxic T Lymphocytes (CTL). High avidity CTL are able to efficiently recognize very low levels of antigen and possess an enhanced capacity to control tumours and viral infection in vivo. 73 domain mutations engineered to knockout the MHC/CD8 interaction have been used to produce CD8 null tetramers. CD8 null tetramers stain immunodominant anti-HCMV (Human Cytomegalovirus) CTL directly ex vivo with equal intensity to wild type tetramers. CD8 null tetramers stain a variety of high avidity CTL clones with similar intensity to wild type tetramers. However, CD8 significantly affects the ability of pMHC tetramers to stain low avidity clones and as a result CD8 null tetramers stain such clones poorly or not at all. In conclusion, wild type pMHC tetramers are able to efficiently stain both high and low avidity CTL and do not enable the assessment of CTL quality. In contrast, CD8 null tetramers stain only high avidity CTL therefore providing a potential tool for identifying such CTL for use in adoptive T cell transfer therapy and a means of assessing the quality of a CTL response directly ex vivo.

OP193

Activation of γδV62 T cells requires contact with a cell of human origin

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Numerous recent studies have shown that the dominant human peripheral blood subset of γδ T cells, which express a γδT2 TCR, are activated in response to low molecular weight nonpeptidic molecules, often of bacterial or parasitic origin. We used IFN-γ ELISPOT to examine the activation of γδ T cells directly ex vivo, polyclonal γδ T cell lines and a γδT2 clone in response to multiple alkyl phosphate, alkylamine, and aminobisphosphonate (nBPs) antigens and purified protein derivative from Mycobacterium tuberculosis (PPD). We show that γδT2 T cells can make IFN-γ in response to all of these structurally diverse antigens. The pharmonetics of responses to alkylphosphate and alkylamine antigens differs from responses to the nBPs. Indeed these different classes are believed to have a different mechanism of action that may explain why nBPs can be pulsed onto APCs. We also demonstrate that a significant proportion of the cells that produce IFN-γ directly ex vivo in response to PPD are γδ T cells. Using several different cell lines of lymphoid and nonlymphoid origin as presenting cells we are able to show that γδ T cell activation requires cell-cell contact with a cell of human origin.

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OP194
Study of CD4+ T-cell–CD8+ T-cell interactions: effect on CD8+ T-cell proliferation
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The interplay between CD4+ T cells and CD8+ T cells in the generation of antigen-specific responses is complex: CD4+ T cells influence CD8+ T-cell immune responses by activating Antigen Presenting Cells (APCs), as well as directly via CD40-CD40L interactions, and by expressing cytokines that influence the behavior of APCs and CD8+ T cells. We have studied the influence of CD4+ T-cell presence on the proliferation of activated CD8+ T cells. We present preliminary data showing that the absence of CD4+ T cells may prevent optimum proliferation of CD8+ T cells. However, in a transwell setting (to prevent direct contact between CD8+ and CD4+ T cells), the presence of CD4+ T cells seemed to result in the suppression of CD8+ T-cell proliferation. Using cytokine bead assays, we found that, in contrast to CD8+ T cells, CD4+ T cells produce significant quantities of both IL-2 and IL-10, cytokines that may influence CD8+ T-cell proliferation. These results may suggest that CD4+ T cells have the capacity both to help as well as suppress CD8+ T-cell proliferation, possibly through the presence of a CD4+ regulatory T-cell population (e.g. CD25+ or CD45RBhigh).

T cell receptors

OP195
Monoclonal TCRs – stable, soluble, high affinity T cell receptors
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The use of soluble T cell receptors (TCRs) as therapeutic targeting agents requires, firstly, the ability to produce stable, soluble TCRs and, secondly, their engineering to have higher affinities for their peptide-HLA ligands.

Vaccines

OP196
Qualitative differences of immune responses induced by modified vaccinia Ankara and formalin-inactivated vaccines in respiratory syncytial virus infection
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Development of vaccines for respiratory syncytial virus (RSV) has been greatly slowed by the tragic outcome of trials of formalin-inactivated vaccines (FI-RSV), which caused severe disease exacerbation in children. This effect has been reproduced in mice, allowing the comparison of the immune and pathogenic effects of new vaccine candidates. Immunizations with Modified vaccinia Ankara (MVA) expressing RSV F or G surface glycoprotein (MVA-F and MVA-G) curtailed virus replication during subsequent pulmonary RSV infection. MVA-F and MVA-G induced both IgG1 and IgG2a, higher IL-12 and IL-18 levels in bronchoalveolar lavage, fewer IL-4/5 and more IFNy producing cells in the lung and did not cause the striking lung eosinophilia induced by FI-RSV sensitization. Nevertheless, immunization with anti-RSV MVA-based vaccines resulted in transient weight loss and respiratory illness upon RSV challenge suggestive of RSV-specific immunopathology. Therefore, MVA-RSV vector vaccines can induce a qualitatively distinct immune response profile that may be advantageous in vaccine development but further studies are necessary in other animal models.

OP197
Effector lymphatic cannulation for the evaluation of immunogeneity of DNA vaccines
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Despite the availability of efficient protocols for mice, DNA vaccination in large animals still gives inconsistent results. We have compared the immune response generated by conventional DNA plasmid vaccination to the one obtained using a bacteriophage delivery system. Both constructs encoded the Hepatitis B surface antigen (HBsAg). The kinetics of humoral and cellular responses to challenge were followed in cannulated lymph of sheep. Cell output was markedly reduced after phage priming. After boosting, there was a partial reduction followed by an increase. With both constructs, there was a reduction in B cell output after priming and an increase after boosting. B cells represented the majority of the blasting cells. The proportion of CD4+ and CD8+ cells increased after priming and boosting whereas γδ TCR +ve cells showed very little variations. Specific antibody responses were detected with both constructs. We will further discuss the relative merits of these different vaccine approaches.
OP198
Immune responses to intranasal immunization with microencapsulated *Yersinia pestis* antigens

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Immune responses in BALB/c mice evoked by a single administration of microencapsulated rF1 and rV antigens from *Yersinia pestis* were investigated. Recombinant F1 and V were individually encapsulated in polymeric microspheres, to the surface of which additional antigen was adsorbed. The microspheres containing either F1 or V were blended and used to immunize mice on a single occasion, by either the intranasal or intramuscular route. Both routes of immunization induced systemic and local immune responses, with high levels of serum IgG being developed in response to both vaccine antigens. In Elispot assays, secretion of cytokines by spleen and draining lymph node cells was demonstrated, revealing activation of both Th1 and Th2 associated cytokines. These results demonstrate that a noninvasive method of immunization such as intranasal instillation of vaccine antigens can provide strong systemic and local immune responses. Microencapsulation of these vaccine antigens has the added advantage that controlled release of the antigens occurs in vivo, so that protective immunity can be induced after only a single immunizing dose.

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OP199
Whole-cell pertussis vaccine protects against * Bordetella pertussis* exacerbated allergic asthma

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The prevalence of asthma is increasing and there has been speculation that immunization may promote allergic sensitization. Previously, we have shown that injection of the respiratory tract by *Bordetella pertussis* exacerbates the allergic asthmatic response. The whole-cell pertussis vaccine (Pw) induces a pattern of immunity similar to infection. We employed a validated murine model of Pw vaccination and pertussis infection in combination with the murine OVA model of allergic asthma to assess the contribution of immunization to the allergic asthmatic response. Although most indices of immune activation are unchanged, we demonstrate that in contrast to infected and sensitized mice, prior immunization with Pw results in significantly reduced IL-10 both systemically and locally. Immunization also reduced the OVA-specific IgE detectable in serum but not other subclasses. Physiological measurements of airway reactivity were also reduced following Pw immunization. Thus immunization with Pw protects against *B. pertussis* exacerbated allergic asthma, despite induction of similar patterns of immunity.

OP200
Safety, immunogenicity and efficacy of a single dose of a pre-erythrocytic malaria vaccine, ICC-1132 formulated in an oil based adjuvant

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ICC-1132 is a hepatitis B core virus like particle, comprised of 240 copies of hepatitis B core protein into which a B cell (NANP) and 2 T cell epitopes of *P. falciparum* circumsporozoite protein have been engineered. The vaccine is formulated in a water-in-oil emulsion, Seppic ISA-720. A single dose of 50 μg was administered i.m. to 11 volunteers. Subjects were challenged with *P. falciparum* sporozoites by 5 infectious mosquito bites 5 to 6 weeks post vaccination alongside 6 malaria naive controls. Local reactogenicity was low with the predominant finding being mild pain at the injection site lasting for 1–3 days. Only 2/11 subjects experienced possibly vaccine related systemic side-effects (fatigue and malaise). The single dose regimen induced anti-NANP antibodies in 10/11 and modest peptide specific responses in *ex vivo* IFNy ELISPOT. All sporozoite challenged individuals developed malaria infection and no significant difference in time to parasitaemia was observed for vaccinees and controls. While this formulation was safe, other formulations and/or multidose regimens will be required to enhance the immunogenicity and efficacy of ICC-1132.

OP201
T cell responses in patients with anogenital intraepithelial neoplasia after vaccination with a recombinant vaccinia virus vaccine (TA-HPV)

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Human papillomaviruses (HPV), particularly types 16 and 18 are the causative agent of Anogenital Intraepithelial Neoplasia (AGIN). HPV E6 and E7 gene expression is necessary for the development and maintenance of HPV transformed cells, representing attractive targets for immunotherapy. TA-HPV is a recombinant vaccinia virus vaccine that expresses HPV16 and 18 E6 and E7. This vaccine was used in a clinical trial to assess clinical and immunological responses in 11 patients with high
grade AGIN. For sensitive detection of low frequency HPV specific T cells, a modified ELISPOT assay was used. Peripheral blood (PBMC) from either healthy volunteers, control AGIN patients or TA-HPV vaccinated patients was stimulated in vitro with HPV16 and 18 peptide pools, before assay for IFN-γ producing cells by ELISPOT. Significant T cell responses against at least one of the peptide pools were seen in healthy volunteers (6/9), nonvaccinated AGIN patients (6/8) and TA-HPV vaccinated patients (8/10). For 2/10 TA-HPV vaccinated patients, boosting of pre-existing T cell responses was seen, while for 6/10 patients novel HPV18 directed T cell responses were detected.

OP201A
Vaccination to protect against Jaagsiekte sheep retrovirus (JSRV) infection and ovine pulmonary adenocarcinoma (OPA)

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JSRV, the aetiological agent of OPA, fails to induce a virus-specific T cell or antibody response in natural infections. In an attempt to induce protective immunity by immunization, sheep were primed and boosted with a recombinant JSRV capsid (CA) antigen, followed by 3 vaccinations with CA-DNA. CA-specific antibodies were detected after the first protein boost and T cell proliferation after the DNA vaccination, although these responses were transient only. Vaccination with a JSRV surface protein (SU) and SU-DNA failed to induce humoral or cell-mediated immunity but a reduced response to mitogen stimulation occurred, suggesting that SU may have immunosuppressive potential. Sheep immunized with whole-inactivated recombinant virus are being monitored currently.

Virology

OP202
Mumps virus induces IL-10

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Despite the extensive use of Mumps virus (MuV) vaccines for almost 40 years, there is still a paucity of knowledge concerning the host-pathogen interaction. The current study was concerned with the humoral and cell mediated responses to attenuated and wild type MuV. Recent work has shown that different haemagglutinins from Influenza virus (HA), or B. pertussis ( FHA) can modulate normal immunity through the specific induction of IL-10. MuV has similar activity due to the the haemagglutinin-neuraminidase (HN) protein. HN is also a subunit vaccine candidate. Therefore we decided to examine these responses further. MuV HN was cloned and expressed in COS-7 cells. We then examined the immune interactions between whole virus or HN and murine T cells or dendritic cells (DC). Immunization of mice with MuV resulted in the preferential establishment of clones with a regulatory phenotype secreting high levels of IL-10. Studies of human and murine DC suggest that HN preferentially induces IL-10, and that this may be a viral strategy for modulating host immunity.

OP203
Foot-and-mouth disease (FMD) emergency vaccine antigen predominantly induces γδ T cell proliferation in naive pigs

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The ‘emergency vaccine’ for foot-and-mouth disease (FMD) can protect animals within 4 days without sufficient neutralizing antibody development or memory T cell responses. Although previous work suggested that FMD emergency vaccine may stimulate innate immune responses and/or pro-inflammatory cytokine production. How emergency vaccine protects animals from full FMD virus challenge in early days of vaccination is not known. Here we demonstrate FMD emergency vaccine antigen (without adjuvant) induces γδ T cell proliferation from naïve pigs, which may play an important role in early protection from FMD virus infection. Naïve pig lymphocytes were cultured in vitro with or without various FMD virus antigen preparations. Emergency vaccine antigen can promote the survival of naïve pig lymphocytes in vitro in an antigen dose dependent manner and also induce lymphocyte proliferation. The majority of proliferating
lymphocytes was defined as γδ T cells by FACS analysis using Ki67 and γδ T cell specific mAbs. Proliferation of γδ T cells from naive pigs was also induced by FMD virus nonstructural proteins but not by purified structural proteins.

**OP204**

**Characterizing the expression of the human cytomegalovirus MHC class 1 homologue gpUL18**


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Human Cytomegalovirus (HCMV) gpUL18 is a MHC class 1 (MHC-1) homologue that associates with α2-microglobulin and binds endogenous peptide. The natural killer (NK) cell inhibitory receptor LIR1/ILT2 was first identified by its interaction with gpUL18 and was found to bind soluble gpUL18 with a 1000-fold higher affinity than classical MHC-1 molecules. The potential role of this glycoprotein in suppressing NK function remains, however, controversial. Our studies identified that the 69 kDa protein conventionally assigned to gpUL18 is expressed together with a novel >100 kDa hyper-glycosylated form. Both forms of gpUL18 could be detected during lytic HCMV infection. Interestingly, although the 69 kDa protein was endoglycosidase H (EndoH)-sensitive and the >100 kDa protein EndoH-resistant, biotinylation experiments revealed that both forms were present on the cell surface. Surface expression of gpUL18 was also observed in TAP-negative fibroblasts suggesting that maturation is independent of normal peptide processing. Data will be presented showing that gpUL18 inhibition of NK cell recognition is found to be dependent on the context in which the gene is expressed.