BRITISH SOCIETY FOR IMMUNOLOGY
&
BRITISH TRANSPLANTATION SOCIETY

1986

JOINT AUTUMN MEETING

Kensington Town Hall & Library
London

November 12, 13, 14

ABSTRACTS OF ORAL PRESENTATIONS (Not for publication)

1. BIOLOGY OF AN I-A$^+$ CELL IN THE T SUPPRESSOR EFFERENT CIRCUIT

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In the TNP (picryl) and phenyloxazolone systems, antigen-specific T suppressor factor acts *inter alia* by arming the T acceptor cell. This then produces a nonspecific T suppressor factor (nsTsF-1) when exposed to haptenized cells. This nsTsf-1 bears I-J determinant(s). The evidence that I-J$^+$ molecules may be a receptor for I-A$^+$ led to the surmise that nsTsF-1 might act through an I-A$^+$ cell. Experiment showed that nsTsF-1 has no effect on the passive transfer of contact sensitivity by immune cells depleted of I-A$^+$ cells by monoclonal anti-I-A antibody and complement. In fact, the cell responsible was an I-A$^+$ T cell, which occurs in specifically immunized mice and liberates a second inhibitor, nsTsF-2 when exposed to nsTsF-1 and (antigen) haptenized cells. This second nsTsF-2 inhibits the passive transfer of contact sensitivity by immune cells incubated in it. NsTsF-2, like nsTsF-1, is antigen nonspecific in its action. However, unlike TsF-1, it carries I-A-like determinants and is MHC restricted in its action on the cells which transfer contact sensitivity. This suggests that nsTsF-2 may interact with an I-A restricted T cell receptor by virtue of its I-A-like determinants.

2. TNP-DEPENDENT SUPPRESSION MEDIATED BY RAT T-CELL-HYBRID-DERIVED FACTORS

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We have isolated rat T-hybrids which secrete a TNP-specific factor that can significantly suppress primed responses to TNP on the carrier protein KLH. Importantly, the KLH-specific response in these cultures is also suppressed. In contrast, responses to a non-cross reactive hapten (LAC) and to KLH after priming with LAC-KLH and exposure to factor, are unaffected. This indicates that factor-mediated suppression can be transferred from TNP to protein carrier. Both overall cell proliferation and differentiation into antibody producing cells are diminished.

We are currently assessing the factors for an ability to suppress rejection of LEWIS kidney allografts by DA recipients which have received TNP-specific factor together with TNP-modified LEWIS antigen.

3. IMMUNOGENETIC EVIDENCE ON THE STRUCTURE OF ANTIGEN-SPECIFIC T SUPPRESSOR FACTOR

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It is known that antigen-specific T suppressor factor (TsF) to TNP (picryl) has a two chain structure - one chain binds antigen (Ag^+), while the other is $I\text{-}J^+$ as shown by absorption with monoclonal antibody. Moreover, there are two I-J (B10.A(3R) and 5R) genetic restrictions in its action: i) in the interchain complementation between the two separated chains needed for biological activity. (As the Ag^+ chain lacks I-J determinants, this implies that the Ag^+ chain has a recognition site for I-J); ii) for interaction with haptenized cells when the T acceptor cell is armed with TsF. The question was asked: which chain carried the recognition site for I-J at stage ii)? Experiment showed that the separated chains from CBA TsF show strict genetic restriction at both stages. However, the Ag^+ chain from (k X b)F1 mice interacted with picrylated cells of both parental haplotypes at stage ii). It was concluded that the Ag^+ chain of TsF has two recognition sites for I-J (or I-J related molecules, e.g. receptors for I-J) - one involved in interchain complementation and the other in the recognition of antigen in the context of I-J.

4. ANTIGEN-SPECIFIC T HELPER FACTOR (ThF) BEARS I-A DETERMINANTS & THEIR ROLE IN THE I-A GENETIC RESTRICTION IN THE ACTION OF ThF

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Antigen-specific T helper factor appears in the culture supernatants of spleen cells from mice treated with cyclophosphamide and then injected with picrylated spleen cells intravenously. It is demonstrated in an "afferent stage assay" by its ability to enable small numbers of picrylated cells injected into the footpads to immunize mice and give rise to contact sensitivity. It practice, picrylated allogeneic spleen cells are used. We have already shown that it has a two chain disulphide bonded structure: one chain binds antigen (Ag^+) while the other chain bears I-A determinants. This communication shows:

i) The $I\text{-}A^+$ chain bears determinants of both the alpha and beta chains of I-A. This suggests that it is coded for by the I-A subregion and is not an "internal image" of I-A.

ii) Two types of ThF occur in (k X d)F1 mice: one bearing $I\text{-}A^k$ determinants and the other $I\text{-}A^d$.

iii) The genetic restriction of ThF in F1 mice depends on the MHC phenotype of the ThF and not on the genotype of the picrylated parental cell used for its induction. This finding is compatible with the view that ThF acts by approximating picryl groups to its $I\text{-}A^+$ chain and hence facilitates recognition by I-A restricted T cell receptors.

5. CONTROL BY H-2 GENES OF MURINE ANTIBODY RESPONSES TO M. TUBERCULOSIS

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The genetic control of antibody responses after immunisation with M. tuberculosis soluble antigens was examined in inbred and H-2 congenic mouse strains. Antibody levels to five distinct epitopes were determined by a competitive inhibition test using radiolabelled murine monoclonal probes. High or low responder antibody levels were associated with either the $H\text{-}2^b$ (TB23, TB71 and TB72 specificities) or the $H\text{-}2^k$ (TB68) allele on both B10 and BALB backgrounds. The high response of TB78 specificity associated with the $H\text{-}2^k$ on the BALB but with $H\text{-}2^b$ haplotype on B10 background. The phenotype of $(C57BL/6xCBA)F_1$ hybrids reflected the high response for four and the low or intermediary response for one (TB68) of the tested paratopes. This is the first demonstration of immune response gene control in respect of defined mycobacterial protein epitopes. The implications towards the analysis of pathogenic or protective mechanisms during mycobacterial infection will be briefly outlined.

6. EVIDENCE FOR IMMUNOREGULATORY CONTROL OF MuLV-INDUCED LYMPHOMAGENESIS BY CLASS II GENES; THE I-A REGION REGULATES BOTH SUSCEPTIBILITY TO LYMPHOMAGENESIS & PHENOTYPE OF VIRAL-INDUCED LYMPHOMAS

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Neonatal infection of C57BL/10 mice with a cloned dualtropic mink cell focus-inducing murine leukemia virus (MuLV), MCF 1233, induces a wide spectrum of lymphomas of T, B and non-T/non-B cell type. In a mapping study using intra H-2 recombinant C57BL/10 mice, we got clear evidence that a) resistance to early development of T-cell lymphomas is

controlled by the H-2 I-1 locus, b) susceptibility to early T-cell lymphomagenesis is associated with an I-A regulated low anti-MCF 1233 envelope antibody response and persistent viral infection of the thymus, c) H-2 I-A^{b} mice, although resistant to early T cell tumours, develop more B cell lymphomas late in life. Possible H-2 class II linked immunoregulatory mechanisms underlying this unique dual influence of the I-A locus on the outcome of MuLV-induced lymphomagenesis will be discussed.

7. EFFECT OF BAN ON THE GROWTH OF TUMOUR CELLS IN VIVO AND IN VITRO

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Four new hybridoma cell lines have been established which secrete BAN (B cell anti 5' -nucleotidase), a 44K protein which acts via 5' -nucleotidase to switch off the production of IgG but not IgM by plasma cells. Two of the new hybridomas also secreted IgM, and two other IgM hybridomas, initially selected for the specificity of their antibodies, were also found to secrete BAN. The growth rates of the two new non IgM secreting hybridomas and two previous non immunoglobulin BAN secreting hybridomas were compared in vivo and in vitro with low BAN secreting control clones. Although the growth rates in vitro varied and were independent of BAN secretion, all the BAN producing clones grew at the same rate in vivo, producing ascitic tumours 2 days before the clones secreting low amounts of BAN.

8. MODULATION OF MURINE T-CELL RESPONSES BY ANTI-IDIOTYPE ANTIBODIES

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Tuberculosis develops despite abundant host T-cell sensitization. Hence it is of interest to dissect the immune repertoire responsible for immune response to myobacterial antigens. We report here that anti-idiotypic reagents represent valid specificity probes for the identification of immunodominant epitopes in mycobacterial infections. Xenogeneic anti-Id sera (Rb71) were raised in rabbits towards a murine monoclonal antibody (TB71) specific to a 38Kd protein antigen of M.tuberculosis. Antisera were made Id-specific by cross-adsorption with normal mouse globulin. This TB71 is located within or close to the antigen-combining site and expressed only on intact TB71 molecules. Affinity-purified Rb71 was studied for its effects on T cell mediated immune responses of mice to a soluble extract from M.tuberculosis (MTSE). Rb71 elicited a

delayed type hypersensitivity (DTH) footpad response and stimulated in vitro proliferative responses of spleen cells from MTSE-sensitised mice. Antisera from three rabbits manifested corresponding activities. The Rb71-responding cells were of the Lyt 1^{+} 2-phenotype. Conversley, Rb71 in incomplete Freunds adjuvant sensitised mice towards DTH and in vitro proliferative responses to the 38Kd antigen. However, injection of Rb71 in the adjuvant, unlike anti-Ids to other mycobacterial MABs, failed to induce an Id^{+} ("Ab3") antibody response. Thus, differences may exist between the "internal image" qualities of anti-Ids in their potential to trigger T and B cell responses.

9. MEMBRANE PROTEASES IN ANTIGEN PRESENTING CELLS

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Antigen processing may occur, at least in part, at the cell surface of antigen presenting cells[1]. In order to analyse in detail the biochemical mechanisms involved, we have measured protease activity in plasma membrane preparations from the antigen presenting B cell lymphoma cell line, A20. At least two enzymes with endopeptidase activity are present, and can be distinguished on the basis of pH optima and inhibitor profile. Data on the initial characterisation of these enzymes will be presented, and their putative roles in the antigen processing will be discussed.

1 Kaye P, Chain BM & Feldmann M (1985) J Immunol. 134, 1930.

10. SYSTEMIC DTH RESPONSES CAN BE SUPPRESSED BY FEEDING ANTIGEN AFTER IMMUNIZATION

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The suppression of systemic antibody and CMI responses by feeding antigen before immunization is a well documented event. Few reports, however, have considered the effect of feeding antigen after immunization, and these have tended to concentrate on the antibody response alone. This study has examined whether DTH responses are susceptible to suppression induced by feeding antigen after immunization.

Oral administration of OVA, one or two weeks after systemic immunization profoundly suppressed subsequent DTH responses upon challenge with antigen. In contrast, antibody responses were unaffected. Furthermore, the extent of suppression was not related to the number of oral doses of OVA given. Finally, cell transfer experiments suggested that this suppression could not be attributed to classical, orally-induced Ts cells, although a population of this sort was generated by feeding the immunized hosts.

11. PROTECTION FROM IMMUNE COMPLEX NEPHRITIS BY SINGLE ORAL DOSES OF ANTIGEN

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Low antibody affinity mice (TO), prone to develop immune complex disease (ICD), were given single oral doses of antigen prior to the induction of immune complex glomerulonephritis by daily parenteral injections of the same antigen. A single dose of oral antigen was capable of conferring almost complete protection from the subsequent induction of IC nephritis. The protection was antigen specific and dependent on the dose of oral antigen administered. Antibody titres were reduced in antigen fed mice. The degree of glomerular immune complex deposition observed correlated with antibody titre. Further experiments, however, are required to ascertain the mechanism involved in protection from ICD by oral immunisation.

12 INABILITY OF INTESTINAL INTRAEPITHELIAL LYMPHOCYTES TO INDUCE SYSTEMIC GRAFT VERSUS HOST REACTION IS DUE TO A FAILURE TO RECIRCULATE IN VIVO

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The majority of intraepithelial lymphocytes (IEL) appear to be T cells, but we have shown that mouse IEL can proliferate in vitro only in the presence of accessory cells or mediators. Here we have examined proliferative functions of IEL in vivo.

CBA IEL could produce a local graft versus host reaction (GvHR) as measured by a popliteal lymph node assay in (CBAxBALB/c)F_1 hosts, but they were incapable of causing systemic lethal GvHR in irradiated F_1 hosts even with the addition of parental or F_1 bone marrow cells.

Although IEL migrated rapidly into collagen gels in vitro, they failed to recirculate into lymphoid tissue in vivo.

Thus, we suggest that the inability of IEL to induce a systemic GvHR is due to a failure to recirculate in vivo.

13.T CELL RESPONSES TO HAPTENATED INSULINS IN $B10^b$ MICE

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$B10^b$ mice produce T and B cell responses to beef but not to pork insulin, which differ from each other by just two amino acids on the A chain. We have studied T cell proliferative responses in these animals to insulin molecules modified by a single trinitrophenyl (TNP) group.

TNP pork insulin primed mice show in vitro responses to TPI, but also to BI and TNP B chain of insulin.

This data suggests that haptenation with TNP converts pork insulin into an immunogenic antigen in $B10^b$ mice.

14. CYTOTOXIC CLONES WITH A FUNCTIONALLY ACTIVE CD3 ANTIGEN THAT LACK THE EPITOPE RECOGNIZED BY THE WT31 MAB, DO NOT SHOW NORMAL PATTERNS OF T CELL RECEPTOR GENE REARRANGEMENTS CODING FOR ALPHA AND ß CHAINS

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All $CD3^+$ $CD4^+$ or $CD8^+$ mature T cells in PBL of normal individuals have been reported to bind the WT31 MAb which recognizes a common determinant on the T cell receptor for antigen (Ti). However, a small subset of normal $CD3^+$ lymphocytes apparently does not bind WT31. In addition, 6 clones were obtained that also lack the WT31 antigen. Like cloned CD2 NK cells, these clones exhibit strong MHC nonrestricted nonspecific cytolytic activity against a variety of tumour target line cells. Although the lytic activity of these $CD3^+$ WT31 clones is MHC non-restricted, the lysis can be blocked like for allospecific cytolysis by anti-CD3 MAb at the effector cell level. Thus, the CD3 antigen appears to be involved in recognition and/or signal processing in these $CD3^+$ $CD4^-$ $CD8^-$ $WT31^-$ clones. By studying rearrange- ment of T cell receptor genes coding for alpha and ß chains we observed that some clones had distinct rearrangement patterns and other clones showed no rearrangements. We conclude that 1) a small subset of normal human $CD3^+$ T cells does not express the common

epitope on the T cell (Ti) receptor (alpha and beta chain), recognized by WT31; 2) these lymphocytes can medicate strong lytic activity against a variety of tumour cells; and 3) the absence of reactivity with WT31 MAb is reflected by this novel detected pattern of rearrangement of the T cell receptor genes.

15. REGULATION OF MAJOR HISTOCOMPATIBILITY COMPLEX CLASS II GENE PRODUCT EXPRESSION BY A MURINE B-CELL LINE

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Mature B-lymphocytes down-regulate expression of Major Histocompatibility Complex (MHC) Class II antigens during differentiation to plasma cells. We have used a murine plasmacytoma as a model to study this down-regulation.

We have found that this cell line expresses MHC Class I but not Class II antigens at the cell surface. Fusion of these cells to activated splenic B-cells yielded only hybrids that expressed MHC Class I but not Class II. Further fusion of one such MHC Class II negative hybrid to a Class II positive B-lymphoma cell line again yielded only MHC Class I positive, Class II negative hybrids. This indicates that the parent plasmacytoma suppresses MHC Class II gene expression by a phenotypically dominant, 'trans'-acting mechanism. Preliminary results indicate that this 'trans'-suppression operates subsequent to MHC Class II gene transcription.

16. A NEW POLYMORPHIC MARKER FOR THE HUMAN T-CELL RECEPTOR ALPHA-CHAIN GENE

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We have analysed the Restriction Fragment Length Polymorphism (RFLP) of the unrearranged human T-CR alpha gene in a panel of 30 normal individuals. Taq 1 digests of DNAs probed with the full length cDNA probe pl.2 revealed polymorphic fragments of 7.0, 2.0 and 1.4 Kb. Using subcloned probes which identify either the V or C segments specifically, the 7.0 and 2.0 Kb fragments were due to polymorphisms of the C alpha segment, and the 1.4 Kb band due to V alpha segment. The C alpha RFLPs were shown by family studies to represent alleles of the C alpha segment, and was due to variation in restriction sites at the 3' end of the gene. The gene frequencies of the two alleles in the study population were also calculated. The V alpha RFLP may represent a variation in numbers of V segments within the specific V gene family.

These new polymorphic markers allow, for the first time, direct genetic analyses of the germ line T-CR, and the influence of such variations on the immune response.

17. TWO DIMENSIONAL IMMUNOELECTROPHORETIC ANALYSIS OF C3d FORMED DURING IN VIVO ACUTE AND CHRONIC COMPLEMENT ACTIVATION

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EDTA plasma samples from 9 pre-term neonates taken before, during, and after culture proven septicaemia, and plasma samples from active SLE and RA patients were tested for C3d.

At least three different forms of C3d may be formed in vivo. The pattern of C3d formed appear to be dependent upon the mechanism of C3 activation. The C3d moieties formed in septicaemic babies and patients with autoimmune diseases will be compared.

The implications of these results for diagnostic assays for C3d and the immunopathogenetic role of C3d will be considered.

18. HLA-A, B AND DR ANTIGENS AND BF AND C4 GENOTYPES IN MULTICASE RHEUMATOID ARTHRITIS & CONTROL FAMILIES

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We have studied C4A and C4B genotypes using immunofixation and haemolytic assay following electrophoresis of desialated plasma or serum in 54 multicase rheumatoid arthritis (RA) (114 RA patients and 290 non-RA relatives) and 24 control families.

We report a reduced frequency of C4BQO in DR4 positive RA patients (9%) as compared to controls (32%) ($p=0.018$).

We also considered C4 genotypes on the most common DR4 positive RA haplotypes. The haplotype B44-C4BQO-BfS-DR4 is eleven times less frequent in RA patients than in controls suggesting for the first time a DR4 bearing haplotype which may exert a protective effect in RA.

19. COMPLEMENT ACTIVATION AND DISEASE ACTIVITY IN RHEUMATOID ARTHRITIS

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Complement (C) activation has been implicated in the pathogenesis of rheumatoid arthritis (RA). Detection of C fragments provides an objective measure of ongoing C activation. To ascertain whether C activation plays a role in the disease activity of RA we measured C3, C4, the fragments C3d and C4d and articular index in 83 patients with classical or definite RA. C3d and C4d were also measured in 30 normal subjects. Both C3d (mean value 139.09 U/ml) and C4d (89.46 U/ml) were significantly higher in RA when compared to controls (C3d 58.1 U/ml, t=6.1347, $p<0.001$; C4d 32.50 U/ml, t=7.4681, $p<0.001$). In contrast, C3 and C4 levels failed to provide evidence of C activation, being normal or increased in all patients. While no correlation was found between concentration of C fragments and disease activity, a positive correlation was observed between the C3d/C3 and C4d/C4 ratios and articular index (r=0.2199, $p<0.03$; r=0.2753, $p<0.01$). These results confirm that both classical and common C pathways are activated in RA and show that the turnover of C factors, as detected by the C3d/C3 and C4d/C4 ratios, is associated with disease severity, suggesting that C activation is directly involved in determining the activity of the disease. The measure of these ratios could provide a useful tool, to monitor disease progress.

20. EFFECT OF RECOMBINANT INTERFERON GAMMA TREATMENT UPON THE SUSCEPTIBILITY OF SFV INFECTED PRIMARY MURINE BRAIN CELL CULTURES TO CYTOTOXIC T-CELL LYSIS

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Recombinant murine interferon gamma (rMuIFN-gamma) was used to enhance major histocompatability complex (MHC) antigen expression in astrocytes derived from baby C3H mouse brains. The rMuIFN-gamma treatment enhanced susceptibility of the astrocytes to lysis by alloreactive cytotoxic T-lymphocytes (CTL). The susceptibility of astrocytes infected with the neurotropic Semliki forest virus (SFV) to lysis by SFV-specific CTL was also enhanced by treatment with rMuIFN-gamma prior to infection: this was despite a very marked diminution of SFV replication and antigen display. We conclude that one role of interferons could be to modulate CTL activity against brain cells via effects on target cell MHC antigen expression.

21. COOPERATION BETWEEN CYTOTOXIC AND HELPER T LYMPHOCYTES IN PROTECTION AGAINST LETHAL SENDAI VIRUS INFECTION

Protection by T cells is MHC-restricted and MHC-regulated, a model for MHC-disease associations.

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C57BL/6 (B7, H-2^b) mice and H-$2K^b$ mutant B6.C-H-2^{bml} (bml) mice differ from each other only in three amino acids in the crucial H-$2K^b$ restriction element for the cytotoxic T (T_c) response against Sendai virus. Bml mice, in contrast to B6 mice are T_c nonresponders against this virus, but show Sendai-specific T-cell proliferation, antibody production and DTH reaction as well as natural killer cell activity, equal to those of B6 mice. B6, Sendai T_c-deficient bml and T-cell deficient B6 nu/nu mice differ from each other in susceptibility to lethal pneumonia induced by i.n. inoculation of virulent Sendai virus. The lethal dose (LD_{50}) in B6 mice averages 152 $TCID_{50}$, in bml mice 14 $TCID_{50}$ and in B6 nu/nu mice 0.5 $TCID_{50}$. The importance of T_c was also demonstrated by the complete protection of B6 nu/nu mice against infection with a lethal virus dose by i.v. injection of a Sendai virus-specific, IL-2 dependent B6.T_c clone. In vivo protection by this T_c clone was H-$2K^b$ restricted.

An important role for virus-specific T_h cells is evident from the difference in susceptibility between bml and B6 nu/nu mice, supported by the demonstration that the survival time of B6 nu/nu and bml nu/nu mice was prolonged by the injection of Sendai-specific B6 or bml T_h clones after a lethal dose of Sendai virus. Strikingly, T_h and T_c cooperate in anti-Sendai virus immunity, since permanent survival of lethally infected nu/nu mice was only achieved by inoculation of a mixture of T_c and T_h clones or a mixture of a T_c clone and recombinant IL-2.

22. ANALYSIS OF VARICELLA ZOSTER VIRUS SPECIFIC CYTOTOXIC T LYMPHOCTYES IN HUMAN PERIPHERAL BLOOD

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The mechanism of T cell control of varicella zoster virus (VZV) infection is unknown. We have investigated whether varicella-zoster virus (VZV) specific cytotoxic T lymphocyte precursors (Tc-P) can be demonstrated in the peripheral blood of seropositive subjects. T cell lines were

established from peripheral blood lymphocytes (PBL) by secondary in vitro stimulation with either heat inactivated cell-free VZV or with VZV infected autologous fibroblasts, and maintained in Interleukin 2. Lines established in response to cell-free VZV were without cytotoxic activity (7/7 donors) and predominatly $T4^+$ (7/7 donors). Lines established in response to autologous infected fibroblasts were predominatly $T8^+$ (7/11 donors), and cytotoxic (8/11 donors), although lines from only 4/11 donors mediated HLA restricted virus specific cytotoxicity. By limiting dilution analysis, the frequency of such VZV specific Tc-P in seropositive subjects tested ranged from <2 - 63 (donor with recent zoster) per 10^6 T cells. We conclude that VZV specific Tc-P are present in normal seropositive subjects, but that their frequency may be lower than that for specific Tc-P against herpesviruses with non-neuronal sites of latency.

23. LIMITING DILUTION ANALYSIS OF HUMAN CYTOMEGALOVIRUS SPECIFIC CYTOTOXIC T CELLS: THE MAJOR RESPONSE IS AGAINST NON-STRUCTURAL VIRUS ANTIGENS

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Virus specific cytotoxic T cells (Tc) are important in recovery both from primary herpesvirus infections and in limiting reactivation of latent virus. We have previously shown that human cytomegalovirus (HCMV) specific Tc precursors (Tc-P) are present in peripheral blood lymphocytes of normal persistently infected individuals without clinical evidence of reactivation. In order to quantitate the number and specificity of such HCMV specific Tc-P, limiting dilution analysis of peripheral blood T cells, using polyclonal and HCMV specific activation, was performed. Subjects with clinical HCMV reactivation and normal latently infected individuals were studied. HCMV specific Tc-P were present at a frequency of 1 in 10,000-20,000 peripheral blood T cells in normal persistently infected individuals. This increased to 1 in 5,000 in a patient who recovered from clinical HCMV pneumonitis. No HCMV specific Tc-P were detected in HCMV seronegative subjects. HCMV specific Tc generated under limiting dilution conditions were assayed against HCMV infected cells expressing either structural (late) or non-structural (immediate early and early) antigens. 60% of all HCMV specific Tc-P recognised the HCMV non-structural antigens. We conclude that the dominant Tc response to HCMV during persistent infection is directed against a non-structural antigen or antigens expressed prior to viral DNA replication.

24. MACROPHAGE ACTIVATION BY FREE OR LIPOSOME-ENCAPSUALTED MTP-PE & THE EFFECTS OF THESE AGENTS ON RESISTANCE TO HERPES VIRUS INFECTION

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The antivial efficacy of liposome encapsulated and free muramyl tripeptide-phosphatidyl ethanolamine (MTP-PE), a lipophilic derivative of MTP, was studied in murine models of herpes virus type I induced pneumonitis and encephalitis. Attempts were made to correlate survival data with changes in macrophage function. Macrophages from peritoneum, lung and liver were examined.

Encapsulated MTP-PE, administered intravenously, was more effective than free MTP-PE in protecting against pneumonitis. MTP-PE protected animals had reduced virus burdens in infected organs, had elevated antibody titres and were resistant to virus rechallenge. In contrast, free MTP-PE was more effective in protecting against encephalitis. In this model, free MTP-PE also caused a reduction in viral burdens of infected organs early in the disease and only latent virus could be rescued from survivors. Furthermore, survivors were resistant to virus rechallenge even though neutralizing antibody levels were low.

Following MTP-PE treatment, peritoneal macrophages exhibited increased phagocytic, tumoricidal, virucidal and bactericidal activities. Liposome-encapsulated MTP-PE was better than free drug in stimulating phagocytic and bactericidal activities whereas the two agents were comparable in stimulating tumoricidal and virucidal activities. Pulmonary macrophages exhibited increased phagocytic and tumoricidal activities following administration of MTP-PE. Once again, encapsulated MTP-PE was more effective than free drug in enhancing these functions. Liver macrophages from free or encapsulated MTP-PE treated mice also showed enhanced phagocytic activity. However, no marked differences were observed between animals treated with free MTP-PE and those treated with encapsulated drug.

In conclusion, it appears that both free and liposome-encapsulated MTP-PE are capable of activating macrophages in various anatomical sites and of enhancing resistance to viral infection. In some instances, liposome-encapsulated drug is more effective in activating macrophages and enhancing resistance whereas in other cases free drug is

superior. The differential effects of free and encapsulated drugs may be inherent in their ability to localize in different anatomical sites and provide local stimulation, i.e., encapsulated MRP-PE localises in the lungs and liver and alleviates pneumonitis, whereas free MTP-PE is known to cross the blood-brain barrier and is more effective in alleviating encephalitis.

25. **ANTIGEN PRESENTATION FOR SUPPRESSOR CELL INDUCTION IN DELAYED HYPERSENSITIVITY REACTIONS TO HERPES SIMPLEX VIRUS**

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Delayed hypersensitivity (DH) to herpes simplex virus type 1 (HSV-1) can be induced in a murine model by infecting mice subcutaneously or epidermally with live virus. By irradiating mice with a low dose of ultraviolet B (UV-B) light three days before infecting with the same dose of live virus DH is suppressed. The suppression of efferent DH is T cell mediated and transferable; two T suppressor cell subsets are found, one $Ly1^+2^-$ and one $Ly1\ 2^+$. UV-B light *in vivo* also alters the handling of HSV-1 antigens by epidermal antigen presenting cells in an *in vitro* antibody primary assay. In this study we have shown that transfer of epidermal cells from mice which had been UV-B irradiated 3 days previously to naive syngeneic recipients at the same time and site as HSV-1 induced suppression of DH rather than DH itself. Transfer of UV-B irradiated epidermal cells at a different site from virus or transfer of normal epidermal cells did not induce suppression of DH. Thus external modulation of local antigen presenting cells could affect the type of immune response generated to HSV-1. This suppression of DH was also transferable and T cell mediated but only $Ly1^+$ $L3T4^+$ $Ly2^-$ T cells were involved.

References:

Howie S, Norval M & Maingay J. J. Inv. Dermatol 86: 125-128 (1986)

Howie S, Norval M & Maingay J. Immunology 57: 225-230 (1986)

Howie S, Norval M, Maingay J & Ross JA. Immunology 58: 653-658 (1986)

Howie S, Ross JA, Norval M & Maingay J. Manuscript submitted for publication.

26. **PHENOTYPIC ANALYSIS OF T SUPPRESSOR CELLS OF DTH TO HSV-1 INDUCED BY UV-IRRADIATED UROCANIC ACID**

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Urocanic acid (UCA), a substance present in the stratum corneum of skin and structurally closely related to histamine [1], has been implicated as the photoreceptor/mediator for UV-B suppression by De Fabo and Noonan [2]. We have previously reported [3] the first direct evidence that the cis-isomer of UCA induces suppression of the delayed type hypersensitivity response to HSV-1 in normal mice. UV-irradiated UCA (23% cis-form) painted on the skin or injected subcutaneously shortly before sensitisation results in suppression which is dose related and of a similar magnitude to that obtained by UV-irradiation of the mice [4]. We now provide evidence that UV-irradiated UCA induces two suppressor cells of identical phenotype to those induced by UV-irradiation of the mice [5]. This lends further support to the hypothesis that UCA is the primary photoreceptor for UV-B suppression in skin.

References:

[1] Morrison H. Photodermatology (1985) 2, 158-165.

[2] De Fabo EC, Noonan FP. J. Exp. Med (1983) 157, 84-98.

[3] Ross JA, Howie SEM, Norval M, Maingay J, Simpson, T. J. Invest. Derm. (1986) (in press).

[4] Howie SEM, Norval M, Maingay J. J. Invest. Derm. (1986) 86, 125-128.

[5] Howie SEM, Norval M, Maingay J, Ross JA. Immunology (1986) 58, 653-658.

27. **CHEMOTACTIC ACTIVITY OF RECOMBINANT HUMAN INTERLEUKIN-1**

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Purified forms of human interleukin-1 (huIL-1) are reported to be chemotactic towards lymphocytes and neutrophils. As microhetergeneity is common in purified IL-1 it has been of value to examine biological properties of the cytokine prepared by recombinant gene technology.

We have characterised the migration of human peripheral blood neutrophils and mononuclear cells in response to recombinant huIL-1 alpha in terms of time course and concentration dependency and demonstrated the true chemotactic nature of this migration. The mechanism of IL-1 chemotaxis differed significantly from that with f-met-leu-phe in that the former was 15- to 20- times more sensitive to the protein kinase-C inhibitor, H7.

28. **INDUCTION OF INTERFERON-GAMMA & INTERLEUKIN-2 MRNA IN HUMAN LEUKOCYTES WITH CALCIUM IONOPHORE AND PHORBOL ESTER**

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The calcium ionophore A23187 and phorbol ester mezerein induced the production of high levels of mRNA from the interferon-gamma and interleukin (IL-2) genes, detected by dot-blot hybridization with cDNA probes.

Message levels of both genes peaked much earlier (<24 hours) than upon induction with conventional mitogens. The presence of cycloheximide, which inhibits protein synthesis by >99%, had no effect on the induction by A23187 plus mezerein of mRNA from either gene, indicating that synthesis of proteins, such as IL-2, is not required for activation of either gene. Macrophage-depleted lymphocytes clearly produced higher message levels of both genes than undepleted cell populations, suggesting that macrophages may to some extent depress gene induction and that macrophage products such as IL-1 are not necessary for induction.

29. **ROLE OF IL-1 AND IL-2 IN SPECIFIC ANTIBODY PRODUCTION BY HUMAN TONSILLAR B CELLS**

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It has been previously demonstrated that IL2 can act as a T cell replacing factor in specific antibody responses to influenza virus by human peripheral blood E-rosette negative (B) cells. However, when tonsillar E^- cells (95% B cells compared with 20% in peripheral blood E^-), were used the response was variable, often being far less than that induced by T cells. In some cases this suboptimal response obtained with IL2 could be increased to the level induced with T cells by the addition of IL1. Large light tonsillar B cells, fractionated on a discontinuous density gradient, responded well to IL2, and did not require the presence of IL1. On the other hand, although small heavy B cells

responded to influenza in the presence of T cells, no response was obtained with antigen and IL2 alone, and little if any synergy was seen with IL1. These findings suggest that small heavy tonsillar B cells may require an additional activation step before they can respond to IL2.

30. **REGULATION OF MEMBRANE IL-1 EXPRESSION ON MURINE B CELLS BY T CELL DERIVED LYMPHOKINES**

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Treatment of B cells with anti-immunoglobulin antibodies (anti-Ig) plus crude T cell derived lymphokine preparations (LK) results in the expression of low levels of a membrane form of IL-1 (mIL-1) within 48 hours. In this system gamma-interferon (gamma-IFN) appears to have a suppressive effect. Culture of B cells with anti-Ig and LK plus monoclonal antibodies to gamma-IFN greatly increases mIL-1. This contrasts with earlier studies showing that gamma-IFN does not downregulate mIL-1 expression on macrophages, but can enhance their expression of mIL-1 induced by other stimuli such as heat killed bacteria. These studies indicate that B cells and macrophages respond to different signals for induction of mIL-1.

31. **INTERLEUKIN 1 AND INTERLEUKIN 2 PRODUCTION BY BOVINE MAMMARY GLAND MONONUCLEAR CELLS**

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The ability of bovine mammary gland mononuclear cells (MGM) to proliferate and produce interleukins 1 and 2 (IL1 and IL2) in response to mitogens and lipopolysaccharide (LPS) was investigated. Although proliferative responses of MGM were considerably lower than those of peripheral blood lymphocytes (PBL), MGM were able to secrete IL1 and IL2 in response to LPS and ConA, respectively.

These studies suggest that monocytes and lymphocytes may play a role in the protection of the mammary gland by the release of interleukins.

32. CYCLOPHOSPHAMIDE-INDUCED EOSINOPHILIA AND CONCURRENT B CELL LYMPHOCYTOSIS IN THE RAT: A MODEL FOR IL-4 GENERATION?

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Cyclophosphamide (Cy) treatment (150 mg/kg) of Sprague-Dawley rats 48 hr before immunization with a T-dependent antigen, ovalbumin, results in striking bone marrow, blood and tissue eosinophilia, maximal at 14 days (from 6 to 50-fold absolute increase in circulating eosinophils)[1] and concurrent with profound lymphopenia. This phenomenon has been tentatively attributed to selective elimination by Cy of T suppressor cells. In this study, T cell subsets, B cells and monocytes/macrophages were enumerated following alkaline phosphatase-anti-alkaline phosphatase (APAAP) staining of mononuclear cells isolated from lymphoid tissue of rats exhibiting eosinophilia. In lymph nodes, a significant increase in the $W3/25^+$: $OX\text{-}8^+$ ratio compared with normal was maintained from days 7 to 14; in the spleen however, this effect was no longer apparent by day 14, due to the emergence of a population of $OX\text{-}8^+$, $OX\text{-}19^+$ large granulated lymphocytes. A 20-fold rise in splenic B cell numbers ($OX\text{-}12^+$) between days 7 and 14 coincided with the eosinophilia. These findings are consistent with the potentiated production of T_H cell-derived soluble factors affecting eosinophil production and differentiation, including possibly, a rat equivalent of interleukin 4 (IL-4) which in the mouse, has been reported to have B cell growth factor activity linked with eosinophilia[2].

1. Thomson AW et al (1986). Scand. J. Immunol. In press.

2. Sanderson CJ et al (1986). Proc. Natl. Acad. Sci. 83: 437-440.

33. ANALYSIS OF mRNA LEVELS OF VARIOUS IMMUNOREGULATORY MEDIATORS DURING AN IMMUNE RESPONSE

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We have used cDNA probes to compare mRNA levels in both mitogen stimulated human peripheral blood lymphocytes (PBLs) and antigen stimulated T-cell clones over a period of 48 hours. Upon stimulation with PHA + phorbol dibutyrate mRNA levels to IL-1 (alpha and β), IL-2, IL-2 receptor and gamma-interferon were found to rise rapidly peaking at about 4-8 hours post-stimulation. While IL-2 mRNA appears to be

strictly controlled, rapidly returning to basal levels, IL-2 receptor levels had not decayed by the end of the 48 hour period. There are differences in both the levels and kinetics of IL-1 alpha and IL-1β mRNA which suggests they may be regulated differently or produced by different cell types.

34. EFFECT OF RECOMBINANT TNF ON THE GROWTH OF UMBILICAL VEIN ENDOTHELIAL CELLS

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TNF has been shown to have multiple effects on normal endothelial cells. We have studied the effect of recombinant TNF on the growth of umbilical vein endothelial cells (HUVEC). Non confluent HUVEC were stimulated to grow by addition of human serum and the proliferative response was assessed by 3H thymidine incorporation. TNF significantly reduced the growth induced by 10% of serum at a dose as low as 0.1U/ml reaching a plateau (60% inhibition) with 50U/ml. This effect could be gradually overcome by addition of increasing doses of EGF. Cytotoxicity was never observed with doses of TNF up to 1000 U/ml.

Conclusion: The inhibitory effect of TNF on growth of endothelial cells could be relevant when TNF is produced in close contact with a proliferating vascular endothelium.

35. TUMOUR NECROSIS FACTOR INDUCTION OF INTERLEUKIN 1

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TNF has been shown to induce collagenase, PGE_2 and to act as an OAF, properties which have also been ascribed to IL1. We have used northern blotting to show TNF can induce the IL1 mRNA.

The induction kinetics for IL1 alpha and IL1β are essentially the same as those seen when PBL are stimulated with phorbol ester and PHA. The IL1β mRNA is the predominant form and can be seen at low levels even in unstimulated cells. Levels of mRNA peaked between 8 and 16 hours and by 48 hours had decreased to nearly basal levels.

36. SEPARATE SIGNALS ARE REQUIRED FOR THE STIMULATION OF HEPATIC SYNTHESIS OF THE THIRD AND FOURTH COMPONENTS OF COMPLEMENT DURING THE ACUTE PHASE RESPONSE

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During the acute-phase response following the intraperitoneal injection of casein into rats, serum levels of C4 peak on day 2 while maximum levels of C3 are reached on day 3. Hepatocytes from rats which have received an intraperitoneal injection of casein two days previously show maximal synthesis of C4 while the synthesis rate for C3 was highest in hepatocytes isolated after three days. Peritoneal macrophages isolated on days 1, 2, 3 or 4 following the intraperitoneal injection of casein were cultured in vitro, and the supernatants harvested. Supernatants from day 2 macrophages selectively stimulated C4 synthesis while supernatants from day 3 and day 4 macrophages selectively stimulated C3 synthesis. Gel filtration chromatography of day 2 macrophage culture supernatants showed a broad peak (40kD-150kD) which stimulated C4 synthesis. Chromatography of the day 4 supernatant showed a single peak (30kD) which stimulated C3 synthesis. Neither peak contained IL-1 activity. Thus stimulation of synthesis of C3 during the acute phase response appears to be mediated by a macrophage secretory product which is distinct from that which stimulates C4 synthesis.

37. PURIFICATION OF A PLASMA PROTEIN THAT INHIBITS COMPLEMENT-MEDIATED PREVENTION OF IMMUNE PRECIPITATION (PIP)

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Normal human serum was passed over IgG-Sepharose, and a peak of protein which inhibited PIP was eluted with guanidine hydrochloride (2 mol.l^{-1}). This inhibitory material was further purified by sequential Protein-A-Sepharose and Con-A-Sepharose chromatography. The final product gave a single band (Mr 60kD) on SDS-PAGE (reduced and non-reduced) with silver staining. The inhibitor is a glycoprotein (PAS staining) with tau-electphoretic mobility, which binds to IgG. A monospecific antiserum to this protein has been produced.

38. PERIPHERAL BLOOD MONOCYTES AND DENDRITIC CELLS SHOW DIFFERENTIAL DQ EXPRESSION

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The expression of high levels of MHC class II molecules by human peripheral blood monocytes (Mo) is well established. However, our analysis of the expression of products of the individual MHC class II subloci revealed that although most Mo expressed high levels of molecules of the DR subtype, only a small proportion of Mo (2-5%) showed strong DQ positivity. Fractionation of Mo populations on the basis of buoyant density and Fc receptor expression indicated that these strongly DQ+ Mo were low density cells and were Fc receptor negative. Furthermore, the strongly DQ+ cells were only weakly reactive with monocyte-specific monoclonal antibodies and poorly phagocytosed latex particles. Populations enriched for strongly DQ+ cells showed a concomitant increase in antigen presenting activity. The absence of typical monocytic characteristics from the strongly DQ+ cells suggests that they may be peripheral blood dendritic cells.

39. IDENTIFICATION AND CHARACTERIZATION OF MACROPHAGE MOLECULES INVOLVED IN THE EXPRESSION OF ACCESSORY CELL FUNCTION

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A large number of monoclonal antibodies (mAb) directed against macrophage surface molecules were screened for their capacity to inhibit accessory cell function. One of the mAb, designated 24, appeared to define a functionally important epitope. mAb 24 inhibited T cell proliferation to the antigens tetanus toxoid and PPD in a dose dependent fashion, but had little or no effect on the response to the mitogens PHA, PWM and OKT3.

The epitope recognised by mAb 24 was found to be present on a large heterodimeric cell surface glycoprotein of M.W. 175kD/95kD which was expressed on a subpopulation of monocytes and most strongly by macrophages in reactive lymphoid tissue. Treatment of monocytes with gamma-interferon (200 u/ml, 18 hrs) greatly increased expression of the antigen. The role of this molecule in macrophage accessory cell function and its possible relation to LFA-1 will be discussed.

40. KINETIC STUDIES ON THE PRESENCE OF ANTIGEN ON LYMPH NODE DENDRITIC CELLS FOLLOWING SKIN-SENSITIZATION

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Dendritic cells (DC) isolated from the lymph nodes of mice 24 hours after skin-painting with the contact sensitizer fluorescein isothiocyanate (FITC) are fluorescent and are capable of initiating immune responses in vivo and in vitro. The time course of changes in the properties of cells isolated from lymph node after skin-painting was investigated from 15 minutes through to seven days. DC in the lymph node retain antigen for up to three days and their functional properties were demonstrated by the ability to stimulate lymphocyte proliferation in vitro and to transfer sensitization for delayed-type hypersensitivity.

41. FUNCTIONAL DEMONSTRATION THAT AFFERENT LYMPH DENDRITIC CELLS ARE INVOLVED IN ANTIGEN CARRIAGE TO A DRAINING LYMPH NODE

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Sheep afferent lymph cells have been collected by the cannulation of a pseudo afferent lymphatic vessel. Cells have been collected before and after an intradermal injection of either PPD or ovalbumin in the area drained by the afferent lymphatic vessels. Cells were fractionated by centrifugation on a discontinuous gradient of Metrizamide to obtain a highly purified population (>95% pure) of MHC Class-II positive dendritic cells. After irradiation, dendritic cells were cultured for five days with either an autologous ovalbumin-specific or PPD-specific sheep T-cell line. Proliferation was measured at the end of the culture by 3H-thymidine uptake. The antigen-specific sheep T-cell lines were generated from peripheral blood mononuclear cells by alternate cycles of antigen-stimulation and clonal expansion with human recombinant IL-2.

Dendritic cells collected 24 and 48 hours after the in vivo antigen-challenge with ovalbumin stimulate proliferation of the ovalbumin-specific cell line but not of the PPD-specific line. This stimulating effect titrates with the number of dendritic cells added to the culture. When the experiment was repeated but using PPD as the antigen, then dendritic cells collected 24 hours after in vivo challenge also stimulated an antigen-specific response. Such that, only the PPD-specific and not the ovalbumin-specific cell line was stimulated to proliferate.

However, dendritic cells collected 48 hours after in vivo antigen challenge with PPD stimulate proliferation of the PPD-specific and the ovalbumin-specific cell line. This non-specific effect by the PPD-pulsed cells is being examined further.

We have recently raised monoclonal antibodies to this population of afferent lymph accessory cells and are currently characterising the identity of the cell type.

42. MIGRATION PATTERNS OF DENDRITIC CELLS (DC) IN THE MOUSE

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DC induce immune response in vitro and they can trigger rejection when administered to engrafted animals. Very little is known about DC traffic in vivo. We have used Indium-111 and Hoescht 33342 labelling to trace highly purified (>99.5%) splenic DC after IV administration. There were no major differences between syngeneic and allogeneic combinations at 3 or 24 hrs after injection. 50-60% of DC were found in the liver, 7-10% in spleen, but none in the peripheral or mesenteric lymph nodes and Peyer's patches. DC appear to accumulate in the T-cell dependent areas of spleen. When DC were administered to nude mice, only ca.3% were recovered from spleen with a concomitant increase in the liver: there was a similar increase in the livers of normal mice after splenectomy. However, migration of DC assumed a normal pattern when nude mice were reconstituted with syngeneic T lymphocytes. The migration of DC vs. T cells into other tissues and at other time intervals has also been examined. From these studies we conclude that (a) DC do not recirculate, (b) T cells may attract DC to the spleen, and (c) there may be an equilibrium between DC traffic to the spleen and liver. Furthermore, (d) DC have a distinct migration pattern: those from mouse spleen behave similarly to those from lymph nodes and to rat DC obtained from the thoracic duct lymph after mesenteric lymphadenectomy and X-irradiation.

43. DENDRITIC CELLS IN HIV RELATED DISORDERS

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The antigen-specific immune response in HIV sero-positive individuals is depressed or absent. This may be due in part to abnormal co-operation between T lymphocytes and antigen presenting cells. Using the method of Knight et al (1986), we have isolated from the peripheral blood of healthy

heterosexuals and patients with persistent generalised lymphadenopathy and AIDS, cells of dendritic morphology. We have examined the expression of various cell surface antigens on these cells. DC express Class II antigens. This expression was shown to be biphasic in controls; the high intensity Class II expression seen on a small proportion of cells from controls and patients with PGL was absent in patients with AIDS. Evidence will be presented that suggests that dendritic cells from patients with PGL are activated *in vivo* whilst those from patients with AIDS are unable to respond to the normal activating signals.

44. RECOGNITION OF SITE ASSOCIATED IDIOTOPES ON A PROTECTIVE MONOCLONAL ANTIBODY TO *PLASMODIUM CHABAUDI* AS BY A POLYCLONAL ANTISERUM

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A rabbit antiserum was produced to a monoclonal antibody NIMP 23 (clone 3). This monoclonal antibody has been shown to react with a 250KD antigen in *P.chabaudi* infected erythrocytes and to confer some protection by passive transfer against homologous parasite challenge[1]. Using the precipitation of radiolabelled parasite antigens, rabbit antiserum to clone 3 (R anti-3) was shown to inhibit the binding of clone 3 to its antigen. This suggests the recognition of site associated idiotopes by the anti-idiotype. A panel of monoclonal antibodies recognizing *P.chabaudi* antigens were examined with R anti-3 to assess whether they bear similar idiotopes. The anti-idiotype is also being used to prime naive animals whose subsequent serum idiotype and protection from infection will be assayed. We have data suggesting that R anti-3 given after infection stimulates idiotype specific T cells suggesting a possible role of this idiotype in B and T cell interactions in *P.chabaudi* infection.

1. Boyle DB et al (1982) Inf. and Immun. 38: 94-102

45. ROLE OF MACROPHAGE ACTIVATING FACTOR & INTERFERON-GAMMA IN RESISTANCE AGAINST EXPERIMENTAL MURINE CUTANEOUS LEISHMANIASIS

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BALB/c mice are highly susceptible to *Leishmania major* infection. They develop disseminating disease with uniform fatality even with a minimal infecting dose of promastigotes. However, the disease progression can be

arrested and complete healing achieved if the mice are sublethally X-irradiated (550 rad) just before infection. Recovered mice develop solid immunity which can be adoptively transferred by Lyt-1$^+$2$^-$ T cells to syngeneic recipients. This report details the study on the mechanism of the cellular immunity. T cells from mice recovered from *L. major* infection were incubated with formalin-fixed promastigotes for 48h *in vitro* and the supernatant assayed for macrophage activating factor (MAF) and the ability to activate *L. major* infected macrophages for leishmanicidal activity. Supernatant from culture of immune T cells contains high levels of MAF and macrophage-activating leishmanicidal activity compared to those from normal T cells or T cells of mice with progressive disease. Recombinant interferon-gamma (gamma-IFN) can also activate macrophages. However, whilst, gamma-IFN activity can be completely abolished by a specific monoclonal antibody, the macrophage activating function in the supernatant of immune T cell culture can only be partially inhibited by the anti-gamma-IFN antibody. These results, therefore, show that both MAF and gamma-IFN may play a causal role in resistance to cutaneous leishmaniasis.

46. ROLE OF GROWTH FACTORS IN CUTANEOUS LEISHMANIASIS

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Antigen primed or mitogen stimulated T lymphocytes are able to synthesize a number of growth factors among which Interleukin-3 (IL-3) has been shown to promote the growth of mast cells, multipotential stem cells and erythroid cells.

We have found that spleen cells from suceptible BALB/c mice with progressive *Leishmania major* infection produce at least five times more IL-3, upon specific antigen stimulation *in vitro*, compared to that by spleen cells from mice immunised with lethally irradiated parasites. The latter mice develop protective immunity, whereas infected animals usually died within four months of infection, bearing large lesions. The role of IL-3 and other growth factors in the outcome of cutaneous leishmaniasis will be discussed, together with the results of studies on IL-3 producer subsets analysed by FACS and the induction of IL-3 responder cells.

47. **CHARACTERISATION OF LEISHMANIA BRAZILIENSIS ISOLATES FROM CUTANEOUS AND MUCOCUTANEOUS LEISHMANIASIS PATIENTS**

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Five L. braziliensis isolated from Brazilian cutaneous leishmaniasis (CL) and mucocutaneous leishmaniasis (MCL) patients were characterised in an attempt to differentiate the two groups. Promastigote surface proteins and protein antigens were identified following lactoperoxidase catalysed radioiodination and 2D-PAGE. Protein and antigen profiles of CL and MCL isolated were very similar. Rabbit antibodies raised against each isolate precipitated approximately 13 labelled antigens. However, only 1 to 6 antigens common to all isolates were precipitated by clinically defined human sera which included a serum of high titre. Immuno-fluorescence analysis using L. braziliensis species and subspecies specific monoclonal antibodies (McMahon-Pratt et al, 1982) confirmed each isolate as L. braziliensis. However, some isolates were reactive with both L. braziliensis and L. b. panamensis specific monoclonal antibodies.

The CL and MCL isolates were differentiated in vivo by their virulence for hamsters. CL isolates produced lesions with pre-patent periods of 13 to 19 days whereas lesions arose from MCL isolates 59 to 107 days post infection. Lesions were monitored histologically and at least three stages of development identified. Late lesions (days 65-7 of development) were histologically negative for Leishmania but were positive by DNA hybridisation using a L. braziliensis KDNA Probe (Wirth & Rogers, 1974).

48. **IMMUNITY TO SCHISTOSOMA MANSONI IN VIVO. DEMONSTRATION OF A CONTRIBUTORY ROLE FOR COMPLEMENT IN BOTH ACQUIRED AND INNATE IMMUNITY**

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In an investigation into the possible role of complement in antibody-mediated resistance to Schistosoma mansoni in rats, C3 levels were depleted by cobra venom factor (CoF)

treatment on day 4, one day before passive transfer of immune serum. It was found that decomplemented rats receiving serum from animals vaccinated with highly irradiated cercariae manifested a 57% increase in worm burden over complement intact controls. However, we also found a 40% increase in worm load in the decomplemented recipients of normal serum compared to complement intact controls suggesting a role for complement in innate resistance. Serum C3 levels in these animals were reduced to 2% of controls after 2 and 4 days and to 10% of normal levels 6 days after CoF treatment.

In addition, the effect of decomplementation on actively acquired immunity was investigated in mice and rats vaccinated with highly irradiated cercariae. By the administration of CoF at different times and using anti-CoF antibody to terminate its effect, we have shown whether complement plays a role in immune attrition in vaccinated and normal rats at the various stages of the parasite's migration through the host.

49. **CLONING-EXPANSION OF ANTIGEN SPECIFIC T CELLS USING ANTI CD3 MONOCLONAL ANTIBODY**

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PBL from a healthy donor were cultured for one week in presence of haemagglutinin and later cloned by limiting dilution. The clones, not yet screened for specificity, were expanded using OKT3 for 4-7 weeks. After this period the cells were challenged with different antigens in a 3 day proliferative assay. 28 clones were tested and 17 (60%) of these showed a specific proliferative response against the haemagglutinin antigen used during the first week. This is the first time that antigen specific clones are expanded in the presence of anti CD3 monoclonal antibody without a loss of specificity. Such an approach could be particularly useful in some circumstances, i.e. autoimmune disorders, where minute amounts of antigen(s) are available.

50. **T CELL RECOGNITION OF SOLID-PHASE ANTIGEN**

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To overcome the constraints of using antigens isolated by monoclonal antibodies in the analysis of cellular immunity to pathogenic organisms we have adopted a procedure involving T cell recognition of antigens fractionated by SDS-PAGE and added to proliferation assays after blotting onto nitrocellulose membranes. Analysis of human T cell responses to M.tuberculosis and M.bovis by this

procedure has revealed distinctive patterns of reactivity to different molecular weight components indicative of selective recognition of immunodominant and species-specific determinants.

51. NOVEL I-A RESTRICTION MECHANISM FOR INFLUENZA HAEMAGGLUTININ SPECIFIC T CELL CLONES

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A panel of I-A^k-restricted T cell clones, specific for variable regions of HA_1 polypeptide of influenza A virus (H3N2 subtype), exhibit a novel class II restriction mechanism for virus recognition.

The clones in association with irradiated F_1 spleen cells ($I\text{-}A^k/I\text{-}A^b$, $I\text{-}A^k/I\text{-}A^d$ or $I\text{-}A^k/I\text{-}A^s$) fail to recognise whole virus but did recognise both purified HA and its tryptic fragments or synthetic peptides of HA. No such difference was evident for recognition of whole virus in association with parental spleen cells or F_1 spleen cells homozygous at the I region (CBA x $B10^1$.AQR (kkkk x qkkd)).

Possible mechanisms for this defect, including antigen processing and quantitative variation of I-A expression will be discussed.

52. MAPPING THE EPITOPE AND AGRETOPE FOR CLASS II-RESTRICTED T-CELL CLONES WITH SYNTHETIC PEPTIDES OF INFLUENZA HAEMAGGLUTININ

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From a panel of influenza virus haemagglutinin (HA)-specific $H\text{-}2^k$ class II-restricted T-cell clones, 6 distinct clones were characterised by their ability to recognise a synthetic peptide corresponding to residues 48-68 of HA1. The response of the clones to a family of synthetic peptides, with amino acid substitutions at sites suggested from the specificity for variant viruses to be involved in recognition, showed that an Asn to Ser change at residue 54 inhibited recognition by each of the 6 clones. Furthermore, an Asp to Asn or Asp to Tyr substitution at residue 63 either shifted the response curve to a higher antigen concentration and/or reduced it to near background level. Responses to the synthetic peptides in association with antigen presenting cells of different haplotypes suggested that the fine specificity of T-cell recognition is determined by residue substitutions at sites critical for interaction with Ia (agretope) or the T-cell receptor (epitope).

53. NICOTINAMIDE PROTECTS TARGET CELLS FROM CELL MEDIATED CYTOLYSIS

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Nicotinamide in concentrations of 5 mM and greater protected virus infected and uninfected ^{51}Cr labeled targets from lysis by natural killer and HLA-DR restricted CD 4^+ cytotoxic T cells. This protection was non-specific in that even spontaneous levels of isotope release from target cells were reduced by nicotinamide. 3-aminobenzamide which, like nicotinamide, inhibits poly (ADP-ribose) synthetase but is not a precursor of NAD, was an effective an inhibitor of target cell lysis while nicotinic acid, an alternative precursor of NAD in cells, was not. Thymidine did not inhibit lysis at concentration up to 20 mM in the presence or absence of nicotinic acid. The data are consistent with the view that a fall in intracellular nicotinamide preceeds the release of ^{51}Cr from target cells damaged by NK or HLA-DR restricted CD 4^+ cytotoxic T cells.

54. T CELL ACTIVATION BY ANTI-IDIOTYPIC ANTIBODY: EVIDENCE FOR THE INTERNAL IMAGE

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Human T cell responsiveness to an anti-idiotypic antibody (anti-Id TB71) correlated with responder and non-responder status to the corresponding 38Kd mycobacterial protein antigen. This finding suggested that the 38Kd antigen and anti-Id TB71 may stimulate related or at least partially overlapping repertoires of T cells. This possibility has implications for the use of anti-idiotypic antibodies as surrogate antigens. Consequently we have examined the mechanism of interaction of anti-Id TB71 with antigen reactive T cells. Both anti-Id TB71 and the 38Kd antigen stimulated T cells through the CD3/Ti receptor complex and required to be presented in the context of MHC Class II molecules on accessory cells. Furthermore, activation by anti-Id TB71 was not achieved by the anti-Id coupled to sepharose beads and was not inhibited by Fc receptor blockade. In additon, T cell responsiveness to anti-Id TB71 was not found to be dependent on the conformational integrity of the antibody binding site. When taken together with the capacity of anti-Id TB71 to stimulate a 38Kd reactive T cell clone these findings are readily explained if the anti-idiotypic antibody contained an internal image of, and can therefore, mimic the antigen.

55. CYTOTOXIC ANTI RAT MHC CLASS II (I-E) MONOCLONAL ANTIBODY: BIOCHEMICAL & FUNCTIONAL DATA

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There is considerable evidence for a role of MHC class II products in the recognition of foreign antigens by T-lymphocytes during allograft rejection. We produced a Mab, 29A1 (IgG1), against the rat class II complex. Results: (1) 29A1 was found to recognize a monomorphic structure of the I-E locus equivalent, identical with that recognized by MRC-OX17. This was demonstrated by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). (2.1) In the mixed-lymphocyte-culture, MLC, addition of purified 29A1 inhibited the proliferation response in a dose-dependent manner comparable to that of MRC-OX17. (2.2) Kinetic studies revealed that 29A1 inhibits MLC response only on days, 0, +1, +2, +3 but not when added on days +4 or +5 (data comparable to MRC-OX6 and MRC-OX17). (2.3) Pretreatment of stimulator cells with purified 29A1 (with/without complement) was inhibitory. The effect was dose dependent. (3.1) In vivo relevance of the above in vitro data was demonstrated in the weakly allogeneic AS ($RT1^a$) LEW ($RT1^l$) system by significant prolongation of skin graft survival. (3.2) Fully allogeneic transplantation of pancreatic rat islets was only successful when graft pretreatment (29A1 plus complement to eliminate "passenger leucocytes") was accompanied by recipient treatment with 29A1. Conclusions: Compared to the commercially available Mab OX17, 29A1 appears to be of equal quality. However, in contrast to OX17 two additional qualities may put it above OX17, namely its cytotoxicity and its availability in an unusual high titre (data presented elsewhere).

56. IN VIVO ADMINISTRATION OF MONOMORPHIC MOUSE MONOCLONAL ANTIBODIES TO RAT CLASS II I-E BUT NOT I-A ANTIGENS SUPPRESSES KIDNEY ALLOGRAFT REJECTION IN THE RAT

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In this study the monomorphic mouse anti rat class II monoclonal antibodies MRC OX6 (anti I-A homologue) and MRC OX17 (anti I-E homologue) were tested for their ability to suppress kidney allograft rejection in rats. Initial *in vivo* titrations established that 1 ml doses of these

antibodies gave free serum antibody levels over the 24 hours following injection. Antibodies were administered intravenously at the time of grafting and on days 1 and 3 after grafting, in the form of immune ascites partially purified by ion exchange chromatography. In the (DA x LEW)F_1 to DA model, one ml doses of high titre MRC OX6 antibody did not suppress rejection. However, the MRC OX17 ascites (with a titre similar to MRC OX6) gave definite suppression of rejection, even in doses as low as 50 µl. A combination of 1 ml MRC OX6 plus 1 ml MRC OX17 was no more effective than MRC OX17 alone. Histologically, MRC OX17 treated rats, even those showing no prolongation of graft survival, showed an altered histological pattern on biopsies taken at day 7, with a more pronounced leucocytic infiltrate and sparing of glomeruli. In the strong DA to LEW model, a combination of 1 ml MRC OX6 and 1 ml of MRC OX17 did not suppress rejection, but there was a slight suppression of the antibody response to the grafts. Within 30 minutes of injection of MRC OX6 antibody, but not following injection of MRC OX17 or other control antibody, the rats developed a syndrome characterised by intestinal hypermotility, excessive secretion of mucus into the gut, and intense inflammation in the region of Peyer's patches and the mesenteric lymph nodes. This susbsided by 24 hours.

57. IDENTIFICATION OF A DISTINCT HLA-CLASS II ALPHA & BETA SUBSET

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In this study we describe the molecular characteristics of HLA-Class II alpha and beta sub-units that appear to be distinct from HLA-DR, -DQ and -DP.

We have used a monoclonal antibody termed EDU-1 which by extensive screening reacts with HLA-Class II structure expressed on cells of different HLA-Class II phenotypes. In addition this antibody reacts with HLA-DR; -DQ deleted mutant cell line, suggesting a possible reaction with HLA-DP - the only apparent HLA-Class II antigen subset retained in such mutant. However, HLA-DP depleted antigen preparations with the antibody B7/21 followed by immunoprecipitation with EDU-1 revealed the presence of alpha and beta subunits of HLA-Class II molecular weight profile. Similar sequential immunoprecipitation experiments with HLA-DR and HLA-DQ monomorphic antibodies L243 and TU22 demonstrate that EDU-1 reacts with a HLA-Class II antigen subset distinct from HLA-DR and -DQ. NEPHGE analysis of the EDU-1 immunoprecipitates revealed the presence of 2 alpha chain spots at approximate isoelectric point pH 5.0. Two beta chain spots focused at pH 7.0 were also detected. These two dimensional profiles were different from those obtained for HLA DR and -DQ.

Additional data shows that the antibody EDU-1 can efficiently block the allostimulation in mixed lymphocyte culture experiments. These data argues for the existence of novel HLA-Class II epitopes not surprising as the HLA-Class II region contains several linked genes that encode the alpha and beta chains of Class II molecules.

58. **DEFINITION OF MULTIPLE POLYMORPHIC EPITOPES ON HLA-CLASS II MOLECULES**

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Monoclonal antibodies (MoAbs) have revealed complexity within the HLA-D region antigens. We have studied epitopes present on a DR3 homozygous cell line using 5 polymorphic MoAbs (NDS9 - alpha DR3; NDS 10 - alpha DR5, <3, <w6; NDS 11 - alpha DR3,5, w6; NDS 12 and 13 - alpha DR3, 5, w6, w8) produced and characterised in our laboratory.

Competitive radioimmunoassays and 2-dimensional gel analyses demonstrated that while the epitopes recognised by NDS 10,11,12 and 13 reside on the same molecule, the epitope recognised by the DR3 specific antibody, NDS 9, is present on a distinctly different molecule.

Thus using MoAbs we have defined multiple epitopes associated with HLA-DR3 with different distributions at the population level.

59. **MAPPING THE HUMAN Hy GENE**

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HLA restricted H-Y specific T cell clones have been used to H-Y type a series of XX males and XY females. Y chromosome specific DNA probes show the majority of XX males inherited certain paternally derived Y chromosome specific DNA sequences whilst XY females show loss of some paternal Y chromosome derived material. Analysis of sexual phenotype, Y chromosome derived DNA sequences and H-Y typing of these sex reversed individuals establishes that the normally Y chromosome associated testis determining gene Tdy is on the proximal end of the Y short arm, whereas the Hy gene is neither on this segment, nor closely linked to it.

60. **EVIDENCE FOR CONTRACTION & DIVERGENCE OF THE HLA-DR SUBREGION**

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During routine typing procedures the individual GER was demonstrated to possess a class II phenotype exhibiting three DR serotypes (DR1,2s,w13). Subsequent family analysis showed that the DR1 and DR2s antigen segregated on one haplotype. RFLP tying revealed that the DR1,2s haplotype contained one alpha and three DR beta chain genes. Biochemical evidence will be presented that this HLA-DR1,2s haplotype codes for one DR molecule carrying the DR1 allodeterminant and two DR2 molecules. Moreover, antigen presentation studies proved that these DR molecules can function as restriction elements.

The data suggest that DR beta chain genes may have been exchanged between diverse haplotypes, indicating that gene expansion or contraction within the HLA-DR subregion does occur. The mechanism that creates a flux of genetic information between different haplotypes and is responsible for the generation of haplotypes with variable numbers of DR beta chain genes may be unequal crossing over.

Part published: Bontrop et al. 1986. J. Immunol. 137, 211-216.

61. **NON-CYTOTOXIC MATERNAL ANTIBODIES TO PATERNAL LYMPHOCYTES DETECTED BY CELLULAR ELISA IN EARLY FIRST PREGNANCY**

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Humoral responses have proved difficult to demonstrate during early human pregnancy. Using a cellular ELISA we have detected maternal IgG antibodies to paternal lymphocytes in sera from 6/20 first trimester primigravidae, 0/15 nulliparae and 5/13 multiparae. Cytotoxic antibodies were detected in 3/13 multiparae but not in any of the other groups.

Family studies demonstrated that non-cytotoxic antibodies in maternal sera were directed to HLA-linked alloantigens. These data support the concept of enhancement as a suppressive mechanism in early pregnancy.

62. EXPRESSION OF CLASS II MHC GENE PRODUCTS BY FALLOPIAN TUBE EPITHELIUM IN PREGNANCY AND THROUGHOUT THE MENSTRUAL CYCLE

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Class II MHC antigens have been identified on various normal and abnormal epithelia but their signficance and function remain unclear. HLA class II antigen expression by fallopian tube epithelium has been investigated in ectopic tubal pregnancy (n=10), in normal early (n=2) and full-term (n=9) intrauterine pregnancy and during the menstrual cycle (n=17). Monoclonal antibodies directed against non-polymorphic (DA6s.231, CR3.43) and polymorphic (DA6.147, DA6.164, anti-leu-10, B7/21) determinants of the HLA-D locus were used in a standard indirect immunoperoxidase method. In ectopic pregnancy, tube epithelium showed uniform, intense reactivity for DR, DP and DQ. A similar reaction pattern was observed in normal first trimester pregnancy. At term, most epithelial cells were DR+, DP+, DQ+, but a few were DP- and DQ-. In fallopian tubes from non-pregnant individuals, a variable number of epithelial cells labelled for DR alpha and DRß but there was never any reactivity for DP or DQ. These results suggest differential regulation of class II MHC gene expression, possibly mediated by hormones and/or a trophoblast product.

63. ATTEMPTS TO IDENTIFY & ISOLATE TROPHOBLAST CELLS FROM MATERNAL PERIPHERAL BLOOD USING THE MONOCLONAL ANTIBODY H315

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The monoclonal antibody H315 is reported to recognise trophoblast in maternal peripheral blood (Covone et al, 1984). Using their method, we have analysed 57 blood samples from 31 pregnant women (6 to 22 weeks'gestation), 10 non-pregnant women and 16 males for H315* positive cells. by fluorescence flow cytometry (FFC) on a Coulter Epics V. High fluorescence (0.2% positive in controls) was found in 0.066 0.002% cells from pregnant and 0.037 0.008% non-pregnant samples (P<0.001). Variably H315 positive cells in maternal blood samples were isolated by cell-sorting. None stained for the trophoblast markers HCG, PAP and HPL. FFC analysis of mixtures of lymphocytes and the H315 positive FL cell-line showed that the FL cells were detectable if present above 1/1000, but not below. It is concluded that H315, as well as reacting with certain trophoblast populations, also recognises a subset of non-placental cells which may be present in the absence of pregnancy.

*Grateful thanks to Professor PM Johnson for supply of antibody, to Professor D Harnden, and to the Kershaw Trust.

64. CLASS I-LIKE MHC MOLECULES EXPRESSED BY TROPHOBLAST

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Human placental villous trophoblast is unreactive with W6/32 mAb, recognizing monomorphic determinants of class I MHC heavy chains, whereas extravillous cytotrophoblast in the placental bed is W6/32-reactive in immunohistology. We have shown that syncytiotrophoblast of baboon placental tissue is strongly W6/32-reactive. Radioimmunoprecipitation and SDS-PAGE has shown that the components recognized have a molecular weight of 41kD and are associated with ß-2 microglobulin. Some human developmental tumour cell lines also show expression of such novel class 1-like MHC molecules. The possible significance for materno-feotal and tumour-host immunological interactions will be discussed.

65. TROPHOBLAST LYMPHOCYTE CROSS-REACTIVE (TLX) ANTIGENS ARE NOT ENCODED BY CLASSICALLY DEFINED HLA A, B, C OR DR LOCI

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Parental disparity for TLX antigens may promote successful pregnancy. Heteroantisera have been described which define a tri-allelic TLX system and another less well defined placental antigen TA1. It is unclear whether either is HLA encoded. 10/10 TLX antisera showed differential activity against T and B lymphocytes from 10 normal donors (ND); patterns of T lymphocytotoxicity corresponded with previously defined TLX groupings. Lymphocytotoxic activity remained unchanged in 6/6 TLX antisera against 6 ND after blocking of lymphocyte Class I HLA antigens with monoclonal antibody (PA 2.6). 10/10 TA1 antisera did show cytotoxicity against B but not T lymphocytes. B lymphocytotoxicity was unchanged in 2/2 TA1 antisera against 6 ND after blocking of HLA-DR antigens with monoclonal antibody (FMC4). Anti-TLX and anti-TA1 antibodies appear not to be directed to HLA,A,B,C or DR antigens.

66. TROPHOBLAST-SPECIFIC GLYCOPROTEIN DEFINED BY MONOCLONAL ANTIBODY 5T4

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The characterisation of a trophoblast antigen, defined by a novel monoclonal antibody (5T4), are described. The normal tissue distribution of the antigen was determined by immunohistology, immunodotting, and immunofluoresence. In formal full term human placenta, 5T4 is strongly expressed only by the syncytiotrophoblast, some extravillous cytotrophoblast and the amniotic epithelium. It is not expressed by maternal pregnancy tissues, nor any other normal tissue tested. Immunoprecipitates of radiolabelled syncytiotrophoblast microvillous plasma membrane (StMPM), identify a glycoprotein of approximately 72kD molecular weight on SDS-PAGE. 5T4 antigen is selectively expressed by diverse tumour cell lines. The characteristics of this onco-trophoblast antigen make it useful as a marker of trophoblast and, potentially, of malignancy.

67. COMPONENTS OF THE LEUCOCYTE INFILTRATION OF HUMAN RENAL ALLOGRAFTS ARE NOT EQUALLY SAMPLED BY FINE-NEEDLE ASPIRATION & NEEDLE-CORE BIOPSY

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In many transplant centres, fine-needle aspiration biopsy (FNAB) has replaced trucut needle-core biopsy (NCB) for the cytological monitoring of graft status. To determine whether infiltrating cell populations sampled by FNAB represent those found in NCBs, we have quantified leucocytes in 21 parallel FNABs and NCBs using a panel of appropriate monoclonal antibodies. Differential leucocyte percentages of antibody stained cells were obtained from FNABs by direct counting and from NCBs by point-counting.

Contaminating blood granulocytes were not counted. Results are expressed as mean % total leucocytes (+/- SEM) and the subgroups in FNABs and NCBs were compared by the Mann-Whitney U test.

	T-lymphocytes	CD4+ Cells	CD8+ Cells	Monocytes/ Macrophages
FNAB	77.9 +/- 14.2	26.4+/-16.6	52.2+/-22.3	22.1+/-14.2
NCB	29.4 +/- 8.7	12.4+/- 5.6	17.0+/- 6.7	71.5+/-5.5

All leucocyte subgroups - FNAb vs NCB : p 0.001

On comparison to NCBs, it is clear that lymphocytes are over-estimated (x2.7) and monocytes/macrophages are under-estimated (x3.2) by FNAB. Blood contamination of FNABs could account for some inaccuracy but even in extreme cases, contamination is less than 20%. It would appear that there are sampling errors due to differences in the aspiration efficiency of different cells. However, these results provide a basis for quantitative correction so that the proportion of cells can be directly related to the true infiltration of the graft. This should enable more accurate studies of graft rejection to be made using FNAB.

68. ULTRASOUND GUIDED BIOPSY OF THE TRANSPLANTED KIDNEY

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Percutaneous renal transplant biopsy is an essential diagnostic tool in the management of acute graft dysfunction. The procedure is not without risk both to the graft and patient. It involves localising the kidney using one of several methods including palpation, metal tagging, fluoroscopy and ultrasound, followed by a vertical pass usually at the upper pole with a tru-cut needle. We describe a technique where real time ultrasound has been used to guide the biopsy needle into cortex alone avoiding the potentially dangerous medullary region. A retrospective study has been conducted on 110 consecutive renal transplant biopsies. 41 of which were obtained under direct ultrasound control and 69 obtained using the vertical pass technique. A total of 70 and 98 cores were obtained respectively and submitted for histology. Renal cortex was present in 84.9% of ultrasound guided cores and 65.6% of vertical pass cores[1]. The average glomerular count was 12 and 5 in each group respectively[2]. Complications included 6 cases of macroscopic haematuria not needing transfusion in the ultrasound guided group and 5 cases in the vertical pass group of which 2 required blood transfusion and exploration of the graft. We believe that ultrasound guided biopsy is a simple and safe procedure producing a significantly higher percentage of adequate biopsies when compared with the standard technique.

1. Chi Square Test $p<0.05$. 2. Mann-Whitney Test $p<0.01$.

69. AVOIDANCE OF SENSITISATION - AN INDICATION FOR TRANSPLANT NEPHRECTOMY?

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Graft failure is a prominent cause of broad sensitisation to HLA antigens; an important risk factor for successful regrafting. We have analysed (retrospectively) the effect of three treatment strategies on panel reactivity in 75 kidney graft failures (68 rejection, 4 vascular, 3 recurrent disease). 21 patients (Group 1) had a failed graft left in situ. 31 patients (Group 2) came to nephrectomy because of graft related symptoms 2 - 180 days after recommencing dialysis. 23 patients (Group 3) underwent early elective nephrectomy while immunosuppressive cover was maintained. Patients sensitisation as determined by lymphocytotoxic panel reactivity was performed in all groups frequently, before, and at intervals after return to dialysis and expressed as the mean of several values during these two, relevant periods. All groups demonstrated a significant increase in panel reactivity associated with transplantation and with graft failure. Group 3 patients undergoing nephrectomy under immunosuppressive cover had a greater chance of remaining unsensitised (66%) than those in groups 1 (31%) or 2 (30%). Of the 52 group 1 and 2 patients whose immunosuppression was withdrawn after failure, 60% (group 2) eventually required nephrectomy. Transplant nephrectomy was safe, 3/54 deaths within 30 days of operation. Consequently we have adopted a policy of early graft nephrectomy with immunosuppressive cover for all failed transplants as they recommence dialysis.

70. FLOW CYTOMETRY CROSSMATCHING IN HIGHLY SENSITIZED RENAL TRANSPLANT RECIPIENTS

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The technique of flow cytometry (FC) has been proposed as a sensitive test for transplant crossmatching. We have therefore evaluated this assay in comparison with the standard cytotoxic crossmatch.

Recipient serum was incubated with donor T lymphocytes. Immunoglobulin bound to the surface of the T cells was then detected by an FITC goat anti-human immunoglobulin, and fluorescence measured using a Cytofluorograf 50L.

Fifty five highly sensitised patients (>90% PRA) were tested by FC, but the decision to transplant was independent of the result. Six patients had a positive FC result with serum

taken on the day of transplantation and five of these grafts failed. The one success was an HLA identical sibling donor in a patient with SLE and autoantibodies. Twenty six patients had a positive FC result with peak samples only, with a 54% one year graft survival. Twenty three patients were entirely negative by FC and their graft survival was 43% at one year.

These data suggest that FC crossmatching in highly sensitised patients does not improve upon cytotoxicity when positive with peak samples; it may predict graft failure when positive on the day of transplantation.

71. GETTING THE BEST FROM A KIDNEY RECIPIENT POOL

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We have shown that 'beneficially' matched grafts (i.e. no HLA-DR incompatibilities, with at most one mismatch on A and B combined) have less than half the risk of failure during the first post-transplant year, than other grafts, even when making the comparison in cyclosporin treated patients (Gilks et al, Traspl. Proc., to appear). In 1984, 24% of donor kidneys exchanged via the UK Transplant Service were beneficially matched. However, computer simulations show that at least 60% of donor kidneys could be beneficially matched with a pool of 3000 patients awaiting kidney transplantation (the current size of the UKTS pool). These simulations incorporate a matching strategy which protects recipients with easily matched phenotypes from receiving non-beneficially matched grafts. As a by-product, this matching strategy permits the calculation of each pool member's expected waiting time to offer a beneficially matched graft. Some implications and examples of this scheme will be presented and compared with conventional schemes.

72. EPIDERMAL DYSPLASIA IN RENAL TRANSPLANT PATIENTS

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Cancerous and pre-cancerous conditions of the skin are frequently seen in renal transplant patients living in sunny climates and has been thought to be uncommon in the UK. We have studied 78 patients (51 males, 27 females) in South Wales who have had functioning renal transplants for periods ranging from 2-20 years. All patients studied had been immunosuppressed with Azathioprine and Prednisolone. A

detailed history was taken with particular regard to sun exposure, and following a detailed examination biopsies were taken from apparently normal sun-exposed skin of the dorsum of the hand. In addition, the presence and type of all skin lesions were recorded, and where possible, biopsies were taken. Premalignant epidermal change (dysplasia) or frank carcinoma was found in 23% (18/78) of the whole group but this finding was restricted to males in the study, of whom 35% (18/51) were affected. In addition, 12% (6/51) of all males had frank squamous cell carcinoma. Males with dysplasia were on average 10 years older than those without this change, and had been transplanted for a mean period of 67 months longer. Eighty seven per cent (14/16) of the dysplasia group had clinical evidence of viral warts compared to 48% (30/62) of patients without dysplasia. Males with dysplasia had spent significantly more time in outdoor occupations than their unaffected counterparts. The presence of premalignant epidermal changes in one third of male transplant patients in the UK is greater than previously reported and is a cause for considerable concern.

73. IMMUNOLOGICAL & ANATOMICAL FACTORS DETERMINE FUNCTION IN SMALL BOWEL TRANSPLANTS

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Without immunosuppression (Lewis x BN)F_1 small intestine allografts (SIA) transplanted (in the form of Thiry Vella fistulae) into parental strain Lewis rats, are rejected in 6 to 9 days; yet survive indefinitely following a short course of Cyclosporin A (CsA). We have investigated the nutrient absorptive capacity of CsA treated SIA by *in vivo* luminal perfusion with isotonic 30mmol l^{-1} glucose/saline. SIA have been compared with isografts and non-transplanted Thiry-Vella loops constructed in littermates. All animals received CsA (15mg/kg/day for 7 days) beginning on the day of surgery. The net flux of water, sodium and glucose was measured at 9 days, and the results correlated with graft weight (table below). There was reduced sodium and water absorption in the transplanted loops compared to the non-transplanted loops ($p<0.01$), but glucose absorption was preserved. Further rats with non-transplanted Thiry-Vella loops, underwent either lymphatic division or autonomic denervation. Both procedures reduced nutrient flux, but autonomic denervation caused severe reduction in sodium and water absorption. Again glucose absorption remained intact.

	n	WATER µ/min/g	SODIUM µ/min/g	GLUCOSE µ/min/g
Allograft	10	38.9±3.4	5.05±0.7	9.1±1.1
Isograft	10	48.9±9.2	5.20±1.9	13.4±2.1
Non-transplanted	6	109±12.1	15.0±1.8	13.3±1.8
Non-transplanted lymphatic division	5	53.9±6.9	7.30±1.2	10.2±1.8
Non-transplanted Autonomic division	5	26.2±5.8	3.61±0.8	9.4±1.7

mean +/- standard deviation

Conclusion: Anatomical as well as immunological factors will decide the successful outcome of small bowel transplantation

74. CYCLOSPORIN (Cy) INHIBITS INFILTRATION BY IL-2R$^+$ CELLS IN RAT RENAL ALLOGRAFTS

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We have investigated cellular infiltration in the DA (RTI^a) to Lewis (RTI^l) and Lewis to DA rat renal allograft models, with appropriate syngeneic controls. Oral Cy was administered in a dose of 10 mg/kg/day for 14 days after transplantation and kidneys were obtained from 100 rats at 3,4,5 and 50 days after grafting. Cryostat tissue sections of the transplanted kidneys were labelled with a panel of monoclonal antibodies, including MRC OX39 (IL-2 receptor) and stained using an immunoperoxidase technique. Cellular infiltration was assessed by point counting and results expressesd as the percentage area of tissue infiltrated by labelled cells (+ SEM). In the DA to LEW model, IL-2R$^+$ infiltration was:-

	DAY 5	DAY 50 (stable)	DAY 50 (rejection)
Cyclosporin	0.5 ± 0.2	0.2 ± 0.2	0.3 ± 0.1
Untreated	6.3 ± 0.4	-	-
Syngeneic controls	.02 ± .02	0	-

These findings demonstrate significant elevation of IL-2R$^+$ infiltration in the untreated (rejecting) allograft model only - for which there is no ethical human equivalent. Thus, while IL-2R$^+$ cells may be indicative of rejection in the untreated rat, the non-elevated levels of these cells in the Cy-modified rejection model at day 50 might suggest that the presence or absence of IL-2R$^+$ cells may not be clinically useful in the diagnosis of human renal allograft rejection.

75. FUNCTIONAL DEVELOPMENT OF THE HUMAN B CELL REPERTOIRE: ONTOGENY OF ANTI-POLYSACCHARIDE RESPONSIVENESS

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The human antibody response to polysaccharide antigens is characterized by the relative late onset in ontogeny: children below 2 years do not respond upon vaccination with polysaccharide vaccines such as Pneumovax. We have previously demonstrated that pneumococcal polysaccharides (PS) behave as human type 2 T cell independent antigens. A specific in vitro anti-PS antibody response can be induced by culturing purified human peripheral blood B cells with 10^{-8} µg/ml type 4 pneumococcal polysaccharides (PS4). The antibody response can be enhanced by the addition of 10% irradiated T cells and B cell growth and differentiation factors (TRF) to the culture. In the latter case antibody-forming cells can be visualised in a so-called spot forming cell (SFC) assay; supernatants of those cultures contain anti PS4 antibodies. Neonatal B cells, obtained from cord blood, do not respond to PS4, neither by generation of SFC nor by specific antibody production. The failure of neonatal B cells to respond to PS4 is not caused by a general inability to induce an in vitro antibody response with these cells because the culture system used does allow the differentiation of e.g. ovalbumin reactive B cells. The negative response of neonatal B cells to PS4 neither seems due to T cell defects: adult T cells are unable to restore responsiveness whereas neonatal T cells support adult B cells to differentiate into anti-PS4 SFC.

In order to address the issue whether PS4 reactive B cells are derived from a particular B cell subset, adult B cells were separated on base of expression of the determinant recognized by the MoAb FMC7. About 40-60% of B cells from adult peripheral blood are FMC7$^+$. While both FMC7$^+$ and FMC7$^-$ B cells respond to polyclonal activation with PWM, the anti-PS4 response is found mainly in FMC7$^+$ B cells. The inability of neonatal B cells to respond to PS4 can however not be attributed to the absence of this particular B cells subset because in cord blood 100% of the B cells carry the FMC7 marker.

76. INDUCTION OF IgE SNYTHESIS BY HUMAN B LYMPHOCYTES

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Studies of Human IgE synthesis have been hampered by the lack of a reproducible model of immunoglobulin production such as exists for the other antibody isotypes. It would appear that IgE production is highly regulated, possibly by isotype restricted T cells and factors, but the exact significance of these agents in vivo is still unclear.

Recent evidence from murine models of B cell activation has indicated that BSF-1, a T,B and mast cell stimulating factor can reduce IgE levels in vivo. We have attempted to establish a human model of B cell activation leading to IgE synthesis. This involved the use of alloreactive T cell clones for cognate T-B interaction, lymphokine preparations and the use of phorbol esters and calcium ionophores to promote B cell activation. The possible role of non isotype specific elements in human IgE synthesis will be discussed.

77. ACTIVATION OF B CELLS FROM PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKAEMIA (CLL) BY THE POLYCLONAL ACTIVATOR, BRANHAMELLA CATARRHALIS

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CLL cells were cultured with two polyclonal B cell activators, Branhamella catarrhalis (Bc) and Staphylococcus aureus Cowan 1 (SAC). Cells from seven of eight patients proliferated in response to Bc, but only one responded well to SAC. Bc also induced secretion of IgM in cells from seven patients; this was unaffected by removal of T cells. When CLL cells were fractionated on density gradients, the greatest immunoglobulin secretion was induced in low density cells; cells of high buoyant density secreted little immunoglobulin. These results demonstrate that Bc is a T-independent activator of both DNA and immunoglobulin synthesis in CLL cells.

78. **POTENTIAL THERAPEUTIC EFFICACY OF HUMAN MONOCLONAL IgG_1 & IgG_3 ANTI-RHESUS D ANTIBODIES**

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Eight monoclonal anti-Rhesus D antibodies have been produced from B-lymphoblastoid cell lines from three individuals[1], and have been characterised for their serological, immunochemical and biological activity.

Anti-D activity was determined by titration, quantitation and specificity, and IgG quantitated by ELISA. IgG subclasses were determined by their reaction with monoclonal antisubclass antibodies and polyclonal antisubclass antisera, SDS-PAGE, absorption by Protein A and Gm allotyping.

The IgG_3 monoclonal Anti-D s were comparable to polyclonal Anti-D s in mediating red cell binding to r-IFN-gamma stimulated monocyte derived cultured macrophages, and were at least 20 times as effective as the IgG_1 Anti D s, suggesting that monoclonal human IgG_3 Anti-D antibodies may be effective therapeutic agents for prevention of hemolytic disease of the newborn.

1 Doyle A, Jones TJ, Bidwell JL & Bradley BA (1985) Human Immunology. 13 199-209.

79. **SPECIFICITY OF ANTIBODIES IN NORMAL HUMAN SERUM TO OVALBUMIN**

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To gain insight into molecular features of allergenic food proteins after absorption from the gut the fine specificity of IgG antibodies in normal human serum to ovalbumin was investigated using ELISA. Preliminary studies with monoclonal antibodies showed that this protein underwent major conformation changes on adsorption to solid surfaces. To preserve the native form on ELISA plates the protein was coupled to the surface via antibody. Serum from ninety-percent of subjects contained IgG antibodies to ovalbumin and adsorption studies with tryptic and CNBr peptides established that antibody activity was specific predominantly for topographic epitopes expressed only on the native molecule.

80. **SPONTANEOUS RELEASE OF FC GAMMA-RECEPTORS FROM HUMAN LYMPHOCYTES IN VITRO**

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Certain normal human peripheral blood lymphocyte membrane receptors for the Fc region of IgG - i.e. Fc gamma-receptors (Fc gamma Rs) appear to be released spontaneously from the cell surface when incubated at 37°C in serum free medium. Kinetic studies showed that Fc gamma R release occurs in two stages. Stage 1 occurs within the first hour of incubation and is probably mediated by proteolytic enzymes. In contrast stage 2 occurs between 2-4 hours and involves active synthesis of Fc gamma Rs. This phenomenon provides a convenient source of soluble lymphocyte Fc gamma Rs by a method which does not require the use of detergent.

81. **HUMAN TRANSFERRIN IS REQUIRED FOR OPTIMAL GROWTH OF HUMAN T LYMPHOCYTES**

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During experiments in which T cell lines were established from rheumatoid synovium it was found that such cells exhibited an absolute requirement for synovial fluid when grown under limiting dilution conditions. Further investigations have shown that the factor within synovial fluid responsible for this effect is human transferrin, and that this requirement is not confined to T cells derived from synovium. When T cells were seeded at 100 cells/well in the presence of feeder cells, IL2, and 10% FCS (100-200 ug/ml bovine transferrin) no outgrowth was observed; consistent outgrowth occurred in the presence of 1ug/ml purified human transferrin. Thus human T cells distinguish between human and bovine transferrin, reflecting the difference in the affinity of human transferrin receptors for these two transferrins.

82. **HISTOLOGICAL HELP**

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The antibody UCHL1 functionally marks T cells capable of providing help for B cell immunoglobulin synthesis (SH Smith et al. Immunology 1986 58 63). It stains 70% of $CD4^+$ cells and 35% of $CD8^+$ cells. Immunoperoxidase staining of tonsil and lymph node shows that histologically it marks T

cells concentrated in and around B follicles. Particular concentrations of positive cells can be found in the periphery of germinal centres. $CD4^+$ and $CD8^+$ cells are by contrast more widespread, with many more positive cells being stained in T zones away from follicles. These results would suggest that T cell help for immunoglobulin synthesis is provided by T cells that enter the B follicles and there stimulate the B cells into division and differentiation to plasma cells.

83. EFFECT OF NEONATAL THYMECTOMY IN MAN

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Children undergoing cardiac bypass operations normally have a near-total thymectomy to allow access to the great vessels. The effect of thymectomy in the very young has not so far been investigated though it is known that splenectomy in childhood results in a partial immuno-deficiency.

Eighteen children who had a thymectomy during open heart surgery when under the age of three months were studied. Each patient was matched for age and timing of operation to two controls, one who had had a thoracic operation with thymectomy and one who had had a minor surgical procedure ('normal children').

Thymectomised patients had lower absolute numbers and proportions of total T cells ($p<0.01$), T4+ve ($p<0.05$) and T8+ve ($p<0.01$) and lower IgA levels than normal children. (Wilcoxon Rank Sum Test for paired data). T cell counts did not correlate with age at operation and did not rise with increasing duration of follow-up. Total lymphocyte count, B cells, IgM and IgG levels were similar in all groups.

Functional studies showed significant reduction in transformation to phytohaemagglutinin and concanavalin A, ($p<0.05$ and $p<0.01$).

84. NOVEL POPULATION OF "RESTING" T HELPER CELLS IN THE NORMAL HUMAN COLONIC MUCOSA

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Double-label immunofluorescence with combinations of monoclonal antibodies was used to study T cell phenotypes and activation states within sections of colonic mucosal tissues. Considering CD3 (UCHT1) as a "pan-T" marker, it was found that only 67.2% (+8.9%) of lamina propria T

lymphocytes co-expressed the CD6 (MBG-6) "pan mature T" antigen, with 82% (+7.4) of these cells being of the CD4+ (helper/inducer) phenotype. Moreover, the T10 activation marker, although expressed in high numbers by other colonic lymphocytes, was present on only 2.7% (+1.4) of the $CD6^+$ subpopulation. Essentially, none of the $CD6^+$ cells expressed IL-2 receptor or HLA-D region antigens. These data suggest that there is a novel population of $CD6^+$ $CD4^+$ non-activated ($T10^-$, Tac^-) or "resting" T helper cells in the normal colonic lamina propria. Whereas the functional significance of the CD6 antigen has not previously been elucidated, our data suggest it may be an important marker of immunoregulatory T cell activity in the large bowel.

85. IMMUNOLOGICAL HETEROGENEITY OF HUMAN GRAFT VERSUS HOST DISEASE

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The number, location and types of leucocytes have been compared in histological sections of skin, liver and large intestine after allogeneic marrow transplantation for leukaemia. In graft-versus-host disease (GvHD), $T4^+$ cells were increased only in the skin whereas $HNK1^+$ cells and macrophages were increased in the skin and liver but not gut. Elevation in $T8^+$ cells were seen in all sites although the activation marker Tac, HLA-DR and T10 were only detectable on cells in the skin. Furthermore, leucocytic infiltration of damaged epithelium was seen in the skin and gut but not the liver despite the expression of epithelial HLA-DR at each site. The cellular composition of GvHD lesions thus varies from site to site and the pathogenic mechanisms may not be the same.

86. MOLECULAR CLONING OF THE HUMAN T-LYMPHOCYTE SURFACE CD2 (T11) ANTIGEN

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The human T lymphocyte antigen CD2 (sheep erythrocyte receptor) was purified from the human T leukaemia cell line J6. Antiserum raised against purified, denatured CD2 identified clones in a lambda gt11 library constructed from J6 mRNA. The cDNA sequence encoded a region that matched all 25 residues of the N-terminal amino acid sequence determined for the purified CD2 antigen. The predicted

polypeptide, of 360 amino acids, contained three potential N-glycosylation sites N-terminal to a putative transmembrane region, and a cytoplasmic domain of 126 amino acids. The extracellular domain of CD2 adjacent to the transmembrane region contained similarities to the corresponding region of the human T-cell antigen T4, and to the immunoglobulin variable kappa chain.

87. **FURTHER STUDIES ON THE PRESENTATION OF ALLOANTIGENS BY HOST ACCESSORY CELLS**

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We have recently shown (Eur. J. Immunol. 16: 569, 1986) that skin allograft rejection is initiated by accessory cells of host origin. The experimental protocol involved the activation of CBA primary (1^{o}) hosts with small numbers of BALB/c spleen cells, the transfer of T lymphocyte-depleted spleen or peritoneal cells 3 days later to secondary (2^{o}) CBA hosts, and the challenge of the latter with BALB/c skin grafts after a further interval of 3 days. The transfer of 5×10^{7} splenic or 3×10^{6} peritoneal 1^{o} host cells resulted in accelerated graft rejection, and we concluded that the cells responsible are non-T, non-B, plastic-adherent accessory cells carrying both host and donor antigens.

We now report further experiments showing that a) the window of antigen presentation is narrow (1-3 days after activation), b) the route of activation determines which lymphoid tissue is most effective in presenting alloantigens, c) skin grafts activate accessory cells in the draining lymph nodes and the spleen on days 3 and 5, respectively, but peritoneal cells not at all; d) cell sorter (Epics V) analysis confirms our previous finding using a double label immunofluorescence technique that 9% of splenic and 14% of peritoneal cells carry both host and donor antigens, and e) depletion of 1^{o} host cells of dendritic cells with a specific antibody and complement prevents 2^{o} host sensitization.

88. **NON RESPONSIVENESS & ANTIGEN PRESENTATION**

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Effective cytotoxic (T_c) cell responses to various antigens are elicited only in mice possessing "responder" alleles at both class I and class II MHC loci. The T_c response against the male-specific antigen H-Y in C57BL/6, (B6, H-2^{b}) is regulated by I-A^{b} and D^{b} molecules.

The bml2 I-A^{b} mutant and the D^{b} mutants bml3 and bml4 are CTL non responders to the H-Y antigen and female mice of these strains do not reject male skin grafts. Previously, we have shown (1) that it was not only possible, using dendritic cells (DC) as antigen presenting cells (APC), to abolish the H-Y specific CTL non responsiveness of the bml2, but also the incapability of male skin graft-rejection of this class II mutant. We now demonstrate that after DC immunization it is possible to restore the capacity of the D^{b} class I mutant bml4 to generate an H-Y specific CTL response, however, without the restoration of the ability to reject male skin grafts. We were not able to overcome H-Y non responsiveness of bml3 mice.

These findings demonstrate again clearly that the CTL response to H-Y is regulated by two separated Ir gene controlled pathways: 1) class II influences are exerted at the level of T helper cells, 2) class I genes regulate the CTL response at the level of CTL precursors. The control of skin graft rejection in these cases remains less clear but possible mechanisms will be discussed.

1. CJP Boog et al., Nature 318: 59 (1985).

89. **MHC ANTIGENS ARE INDUCED TO A COMPARABLE LEVEL IN REJECTING & NON-REJECTING RENAL ALLOGRAFTS**

KJ Wood, MJ Dallman & PJ Morris

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Preoperative transfusion with donor blood results in long-term renal allograft survival in two rat strain combinations, DA-$RT1^{a}$ to PVG-$RT1^{c}$ and LEW-$RT1^{l}$ to DR-$RT1^{a}$. In untreated recipients renal allografts undergo irreversible rejection within 10 days. The kinetics of induction of class I and class II MHC antigens in transplanted kidneys from transfused and untreated recipients were studied. Polymorphic monoclonal or polyclonal antibodies specific for donor class I or class II MHC antigens were used for quantitative absorption analyses. In addition, antibodies against rat leucocyte subsets were used for immunohistology.

The amount of class I and class II antigen induced on grafts from transfused animals was equal to or greater than that expressed by rejecting grafts analysed at the same time after transplantation. Phenotypic analysis of cells revealed no clear difference in the magnitude or composition of cells infiltrating rejecting and non-rejecting grafts. However the percentage of cells expressing the IL-2 receptor was much lower in grafts from transfused recipients than in those from untreated animals.

Thus the prolonged survival of renal allografts in transfused rats cannot be explained by a lack of induction of target antigen on the kidney for either class I or class II directed effector cells.

90. RAT HETEROTOPIC HEART TRANSPLANTATION: SERIAL FREQUENCY ANALYSIS OF PRECURSOR DONOR-LYTIC CYTOTOXIC LYMPHOCYTES

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A series of heterotopic cardiac allografts were established using PVG (RTI^c) donor and LEWIS (RTI^1) recipient rats. The mean period between implantation and complete functional rejection was 5.93 + 0.70 days (mean + s.d.; n = 15).

Recipient animals were killed daily between days 0 and 7 after transplantation and samples of myocardial tissue were removed for histological examination. At each time point lymphocytes were recovered from peripheral blood and disrupted graft tissue and the frequency of precursor donor-lytic cytotoxic lymphocytes (pCTL) in each population was determined by limiting dilution analysis.

Infiltrating mononuclear cells were first observed within the myocardium of the graft on days 1-2 after transplantation and reached peak numbers by days 3-4; however, extensive myocyte necrosis was not apparent until day 5.

During the first 3-4 days post-operation the frequency of pCTL in the peripheral blood showed a drop from the pre-operative value of 1/30,300 but then increased rapidly to reach values up to 1/3,400 by day 7. Precursor CTL were first detected within the graft-infiltrating cell population on day 2 post-operation and reached maximal frequencies of up to 1/4,800 by day 5.

In conclusion, measurement of the frequency of pCTL within peripheral blood or intra-graft cell populations can provide a quantitative correlate with histopathological evidence of cardiac allograft rejection.

91. DONOR SPECIFIC BLOOD TRANSFUSION PRIMES THE HOST FOR THE GENERATION OF SPECIFIC CYTOTOXIC CELLS

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Pretreatment with donor blood specifically prolongs the survival of renal allografts in the rat. We have investigated the effect of blood transfusion on the generation of specific cytotoxic cells. Lymphocytes (lymph node, spleen or TDL) from transfused rats were cocultured with irradiated blood donor-specific or third party allogeneic lymph node cells in MLC. The activity of generated cytotoxic cells against blood donor and third party Con A targets was measured in a ^{51}Cr-release assay.

Restimulation in vitro of lymph node effector cells by blood donor-specific stimulators results in markedly increased levels of specific cytotoxicity in contrast to the same cells restimulated with a third party alloantigen. Thus prior blood transfusion would appear to prime the recipient against donor histocompatibility antigens allowing the generation of donor-specific cytotoxic cells after restimulation in vitro.

92. SPECIFIC CYTOTOXIC EFFECTOR CELLS ARE FOUND IN NON-REJECTING RENAL ALLOGRAFTS IN TRANSFUSED RATS

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Donor specific blood transfusion (DST) may prolong rat renal allograft survival indefinitely. Following DST and renal transplantation in both the LEW-RTI^1 to DR-RTI^a to PVG-RTI^c strain combinations, high levels of donor alloantigen specific cytotoxic effector activity was observed within the grafted kidney (18% and 39% at effector: target ratio 100:1 vs 36% and 32% in untreated controls respectively). Further, a kinetic analysis demonstrated that these cytotoxic cells infiltrated the transplant earlier in transfused animals than in untreated controls. Thus firstly, cytotoxic effector cells may be present in a renal allograft that is not rejected and secondly, donor specific blood transfusion induces an accelerated immunological response to the subsequent renal allograft.

93. **INFLUENCE OF MHC SUBREGION DIFFERENCES IN ORTHOTOPIC RAT LIVER TRANSPLANTATION**

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The influence of the MHC subregions A, C (class I) and B (class II) on the immune response after allogeneic orthotopic liver transplantation in congenic rat strains was investigated by analysis of survival times, graft histology and induction of class II antigen expression on Kupffer cells. Donor-recipient combinations with one, two or three subregion differences were examined in both high and low responder groups. Allograft survival was mainly influenced by the responder status. However, in the high responder group class II (RTI B) mismatch caused the strongest alloresponse. Graft histology correlated with the survival times and class II antigen expression paralleled the immune response.

94. **BULK PURIFICATION OF A NATURALLY OCCURRING SOLUBLE FORM OF RTI-A CLASS I MHC ANTIGENS FROM DA RAT LIVER, AND STUDIES ON SPECIFIC IMMUNOSUPPRESSION.**

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We describe the bulk purification of a water soluble form of RTI-A class I antigens from aqueous extracts of DA liver. Using a combination of monoclonal antibody affinity, lentil lectin affinity and gel permeation chromatography, we were able to obain large quantitities of pure water soluble RTI-A antigens. Typically, from 40 DA liver, 0.5 mg of pure antigen with antigen activity equivalent to 7×10^9 nucleated DA spleen cells was obtained. The water soluble RTI-A molecule had a discrete heavy chain of 40 kD, linked non-covalently to β2 microglobulin. The heavy chain of the water soluble RTI-A molecule was 5 kD smaller than the membrane-bound form of RTI-A from DA liver membranes. The smaller molecule almost certainly represents a secreted form of RTI-A class I molecules which lack the transmembrane domain (exon 5). An identical water soluble class I molecule could be purified from DA kidney and serum. The half life of the water soluble RTI-A molecule in serum was measured at 1.5 hours. However, large quantitiies of the water soluble RTI-A class I antigen from the DA strain, given intravenously to PVC recipients of DA cardiac allografts by a variety of protocols, did not have any effect on graft survival. The fairly ready availability of mg quantities of pure class I transplantation antigens should be of considerable value for studies in transplantation.

95. **MEMBRANE FRAGMENTS ARE LESS EFFECTIVE IN THE SUPPRESSION OF ALLOGRAFT REJECTION IN THE RAT THAN WHOLE CELLS**

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Donor LEW spleen lymphocytes can be used to induce specific unresponsiveness to LEW kidney allografts in the DA rat. This response is donor specific as third party BN cells are ineffective (Median survival time (MST) 10 days), and dose dependent, (10^6 cells or more are effective, MST >100 days, 10^5 cells or less are not, MST 10 days). Homogenised or sonicated the spleen cells induced suppression of rejection in doses equivalent to 5×10^7 viable spleen cells (as determined by a quantitative absorption analysis of the amount of Class I and Class II MHC antigen present) (MST >100 days). 10^6 cell equivalents were ineffective (MST 10 days), while 10^7 cell equivalents were only partially effective, three out of five animals rejecting at 10 days.

Thus although sonicated and homogenised cells can cause suppression of allograft rejection, a 50 fold higher dose is required than when whole viable lymphocytes alone are used. This may be a reflection of the localisation and processing of antigen derived from cellular fragments in contrast to antigen presented on the surface of whole cells.

96. **CORRELATION OF RENAL ALLOGRAFT SURVIVAL AND MIXED LYMPHOCYTE CULTURE RESPONSES IN TRANSFUSED RATS IS DEPENDENT ON THE SOURCE OF RESPONDING CELLS**

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Donor-specific blood transfusion prolongs renal allograft survival indefinitely in some rat strain combinations. To investigate the mechanism of this 'transfusion effect' unidirectional MLR's between transfused animals and their specific blood donor or third party lymph node cells were performed under conditions defined by kinetic analyses. Responding cells were of lymph node, spleen or TDL origin. Lymph node and TDL cell proliferation, as measured by ^3H thymidine uptake, was markedly reduced when stimulation was provided by blood donor specific lymph node cells as compared to the response of the same cells to a third party strain. In contrast spleen cells showed an increased proliferative response against the same blood donor-specific stimulating cells.

97. BLOOD TRANSFUSION EFFECT IN THE MOUSE

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There is solid evidence that blood transfusion has a favourable effect on the survival of renal transplants. However the mechanism underlying this effect is not known. In this study we used the mouse as a model to investigate the transfusion effect. C57 BL/6 (B6, H-2^b) mice were injected i.v. with $5x10^7$ spleen cells derived from bm1 (H-$2k^b$ class I MHC mutant difference), bml2 (H-2 I-A^b class II mutant difference or (bml x bml2) F_1 mice. B6 recipient mice received three i.v. injections with an interval of one week. The effect on alloantigen specific T-cell reactivity of this immunization schedule was measured in vivo by determination of skin allograft rejection and allo DTH reaction and in vitro by determination of the alloantigen specific CTL precursor (CTL_p) frequency. The results indicate that after transfusion of spleen cells across a bml class I MHC mutant, bml2 class II MHC mutant or (bml x bml2)F_1 class I and class II MHC mutant difference the alloantigen specific DTH reaction in B6 mice is completely suppressed. However, with respect to skin allograft survival only transfusion across a bml class I MHC mutant or bml2 class II MHC mutant difference significantly prolongs skin allograft survival whereas transfusion across a (bml x bml2)F_1 class I and class II MHC mutant difference has no effect. This corresponds with the fact that the alloantigen specific CTLp frequency is not changed by transfusion of F_1 cells but a severe depression of the alloantigen specific CTLp frequency was observed in B6 recipients transfused with bml cells.

In conclusion it is possible to induce a transfusion effect across a class I or a class II MHC mutant difference but not across a combined class I and class II mutant difference.

The CTLp frequency after transfusion inversely correlates with skin allograft survival whereas the allo DTH reaction does not show such a correlation.

98. CLASS II MISMATCHES AT THE DNA LEVEL IN SEROLOGICALLY COMPATIBLE HUMAN RENAL TRANSPLANTS

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Despite advances in immunosuppression and the beneficial effects which some centres show for DR matching, serologically compatible kidneys may still be rejected. We have examined 16 DR matched donor recipient pairs where the graft has suffered irreversible rejection and 6 controls with successful grafts, by Southern blotting and hybridization with a number of gene probes. Our results demonstrate that it is possible to detect Class II mismatches at the DNA level which remain undetected by serology. In some cases mismatches are for DR B and DQ B while in others, DQ B incompatibilities alone are observed.

99. DR, DQ AND DR ALPHA RFLP ASSOCIATIONS IN COELIAC DISEASE (CD)

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Many reports have established that the DR3 and DR7 are increased in CD but that DQw2 may be the primary marker. We have extended our serological studies to the genomic level by using Southern blot analyses to identify RFLP in 86 control individuals and 46 patients with CD. In this report we compare DR alpha RFLP in association with DR and DQ serological types in the two groups. Using the DR alpha cDNA probe with restriction enzyme Bgl II, three allelic fragments - 4.5, 4.2 and 3.8 kb - were identified which when correlated with DR typing indicated heterogeneity for DR3, DRw6 and DR7. 90% of all DR3 individuals had the 4.2 kb fragment and the 4.5 kb fragment was present in the remainder. There was no difference in their distribution in the two groups. DR7 individuals had either the 3.8 or 4.5 kb fragment. The 4.5 kb fragment was found in 8/14 (57%) controls and 15/17 (92%) patients with CD ($p<0.05$). Those who were DR7 and 4.5 kb were all Dqw2 and those that were DR7 and 3.8 kb were all DQw3. We have now constructed a series of preferential allelic associations incorporating HLA-B, DR, DR alpha, DQ polymorphisms for the DR7 associated disease susceptibility gene for CD: (B44)-DR7-DQw2-DR alpha 4.5 kb.

100. HLA IN CHRONIC PANCREATITIS

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Histocompatibility antigens were studied in 50 caucasian British subjects (29 male) with well defined chronic pancreatitis. Cases with aetiological factors other than alcohol and insulin-dependent diabetics were excluded. In

22 patients (20 male) mean weekly ethanol intake was more than 100g (in most cases substantially so); the remaining 28 had idiopathic chronic pancreatitis (ICP). There were overall excesses of B44, CW5 and DR4 but these were not significant when corrected for the number of antigens tested. In ICP there were no HLA associations but in those with alcohol-related disease the excesses of B44 (54.5%, control 29.4%) CW5 (54.5% v 15.9%) and DR4 (61.1% v 33.6%) were significant ($p<0.01$, 0.0005, 0.025 respectively) and that for CW5 remained so after correction ($p<0.05$). These results favour an hypothesis that hereditary factors are important in the aetiology of chronic pancreatitis.

101. GENETIC MARKERS ASSOCIATED WITH GRAFT-VERSUS-HOST DISEASE IN HLA-IDENTICAL MARROW TRANSPLANTATION FOR LEUKEMIA

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To identify genetic loci linked to putative "minor" non-HLA histomcompatibility antigens involved in graft-versus-host disease (GVHD), the association between 22 polymorphic blood genetic markers and GVHD was studied retrospectively in 543 leukemic patients and their HLA-identical sibling donors. Patients with recent transfusions were excluded. The genetic markers included 6 red cell antigens, 8 red cell enzymes, and 8 serum proteins. Phenotypically identical patient/donor pairs were compared with phenotypically non-identical pairs for the incidence of GVHD. In univariate analysis, none of the 22 markers showed an association with acute GVHD, and 3 markers (Rhesus, MNSs blood group and acid phosphatase) showed a significant association with chronic or de novo chronic GVHD. For Rhesus and MNSs, the association was more pronounced when genotypically identical pairs were compared with non-identical pairs. The effect of the 3 markers was cumulative: pairs with all 3 markers identical had the lowest incidence of chronic and de novo chronic GVHD (32% and 24% respectively), pairs with 1 marker different a higher incidence (44% and 34%), and pairs with 2 or 3 markers different the highest incidence (both 54%). Neither Rhesus nor MNS bloodgroup by itself was involved as a "minor" antigen. In logistic regression analyses, phenotypical identity for all 3 markers remained signficantly associated with a decreased risk for chronic ($p=0.02$) and de novo chronic GVHD ($p=0.003$). The combination of Rhesus and MNSs showed a weak association with acute GVHD ($p=0.08$) and a significant association with acute and chronic GVHD ($p=0.008$).

These findings present evidence for at least 2 or 3 "minor" non-HLA histomcompatibility antigens involved in GVHD.

102. EXPRESSION OF HISTOCOMPATIBILITY ANTIGENS AND CHARACTERISATION OF MONONUCLEAR CELL INFILTRATES IN HUMAN RENAL CELL CARCINOMAS

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Neoplastic tissue was obtained at operation from 10 renal cell carcinomas, from the adjacent normal kidney in 6 cases and from one other normal kidney. The biopsies were snap frozen in liquid nitrogen and sections were subsequently stained with monoclonal antibodies against major histocompatibility complex antigens, Class I and II, and several types of mononuclear cell, by the indirect immunoperoxidase method. The degree of staining was graded from heavy 4, through moderate 3, few 2, occasional 1, to nil 0. MHC Ag were consistently expressed, grade 2-4, by the glomerular basement membranes and proximal convuoluted tubules of normal kidney, but were absent in 8 of 10 carcinomas. There was a grade 3-4 mononuclear cell infiltration in the stroma of normal kidney and between the carcinoma cells which was composed principally of macrophages. However, in the two carcinomas expressing MHC Ag there was a grade 2-3 infiltration with T lymphocytes. The absence of MHC Ag on carcinoma cells mitigates against attempts to potentiate the patient's immune response to his tumour, eg by renal artery embolization.

103. MHC CLASS II ANTIGEN AND IMMUNOGLOBULIN EXPRESSION IN PHENOTYPIC VARIANT SUBLINES OF THE BURKITT'S LYMPHOMA CELL LINE NAMALVA

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A number of spontaneous phenotypic variant sublines of Namalva were examined with cDNA probes for the different MHC class II beta genes and with mAbs specific for the corresponding cell surface antigens (DP, DQ and DR antigens). Like other malignant B cells* some of the Namalva sublines expressed MHC class II antigens in a non-coordinate manner: In some cases DR antigens were expressed at the cell surface but DP and DQ antigens were not detectable. Other sublines had lost all MHC class II expression. The different sublines had identical DNA restriction fragment patterns when analysed with the MHC class II-specific probes. The ability to express Ig heavy chain correlated with the ability to express MHC class II antigens: where MHC class II expression was lost, so was Ig expression. In sublines expressing high level of MHC class

II antigens here was a high rate of Ig secretion. The correlation between Ig and MHC class II antigens suggests that there may be common mechanisms regulating the expression of these two regions.

*Guy, Krajewski & Dewar (1986) Br. J. Cancer 53, 161

104. EXPRESSION OF MHC CLASS I GENES IN EMBRYONIC AND EXTRA-EMBRYONIC TISSUES

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The developing mammalian embryo fails to be rejected by maternal immune responses directed against paternal allo-antigens. One possible explanation for this is that embryonic genes encoding paternal MHC alloantigens are not expressed in embryonic or extra-embryonic tissues during gestation.

We have used a highly sensitive and specific method for detecting maternal or paternal MHC transcripts in embryonic and extra-embryonic tissues. Our data indicates that while paternal MHC genes are transcribed at low levels in extra-embryonic tissues at day 14 of gestation in mice, transcription of maternal gene is significantly higher. This suggests that transcription of maternal and paternal MHC genes is differentially regulated during development.

105. TRANSCUTANEOUS MEASUREMENT OF pO_2 AND pCO_2 IN THE DERMIS AT THE SITE OF THE TUBERCULIN TEST IN HEALTHY SUBJECTS

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Of 9 healthy subjects skin tested with tuberculin PPD, 6 developed induration, 3 showed erythema and 1 was negative. $TcpCo_2$ was raised in all subjects, usually maximal at 48 hours; this local acidosis explains partly the fall in dermal pH reported previously. A fall in $TcpO_2$ was seen at 48-96 hours in positive reactors, profound in 4 subjects: the negative reactor had a rise in $TcpO_2$. The rate of rise in $TcpO_2$ at the test site after inhalation of 100% O_2 was increased in some positive reactors, but not others. These measurements indicate that in intense reactions the increased dermal blood flow noted by laser-Doppler velocimetry is not necessarily enough to compensate for the increased metabolism of the infiltrating lymphoctyes and monocytes.

106. PROCESSING & PRESENTATION OF MYCOBACTERIAL ANTIGENS BY HUMAN MONOCYTES

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The ability of monocytes from healthy BCG vaccinated individuals to process and present both soluble and particulate mycobacterial antigenic preparations of *M.tuberculosis* and *M.leprae* is assessed by an *in vitro* T cell proliferation assay. Killed whole organisms were found to have a weak stimulatory effect which could be enhanced by ultasonication. The concentration-dependent bimodal effect of the antigens is demonstrated.

Studies using radiolabelled antigen (both whole organisms and soluble extracts) indicate that uptake is rapid. Using monocytes that have been pulsed and fixed, and by using lysosomotropic agents and specific protease inhibitors we have shown that processing of all of the mycobacterial antigens used probably requires a lysosomal pathway. We have also investigated the fate of radiolabelled antigens and the nature of their catabolic products.

107. LIMITING DILUTION ANALYSIS OF THE HUMAN T CELL RESPONSE TO MYCOBACTERIAL ANTIGENS

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Limiting dilution analysis (LDA) was carried out to determine the frequencies of human T lymphocytes responding to soluble mycobacterial antigens from *Mycobacterium tuberculosis* purified protein derivative (PPD) and *Mycobacterium leprae* (MLS). Antigen-induced lymphocyte activation was measured by means of [^{3}H]-thymidine incorporating on day 10 of culture in the presence of suboptimal concentations of IL-2. In healthy BCG vaccinated individuals from the UK the frequency of T lymphocytes responding to PPD was 1.5 to 4 times greater than to MLS. Frequencies between 1/1970 to 1/13982 were observed in response to PPD and between 1/4097 to 1/24717 in response to MLS. A proportion of T lymphocytes in the peripheral blood also showed responses to suboptimal concentrations of IL-2 alone with frequencies ranging from 1/15635 to 1/127,053.

In leprosy patients relative frequencies of PPD and MLS reactive T cells varied depending on the type of leprosy. In tuberculoid individuals the frequency of MLS reactive T lymphocytes was either greater than or similar to the frequency of PPD reactive T cells. In lepromatous patients,

heterogeneous responses were observed with some patients showing no M.leprae reactivity whereas others exhibited M.leprae reactive T cells in the presence of exogenous IL-2. In contrast, PPD reactive T cell frequencies were similar in both the tuberculoid and lepromatous groups.

These studies demonstrate that LDA may be a useful means of quantifying the immune status of leprosy patients and for investigating the mechanism of unresponsiveness in lepromatous leprosy.

108. MOUSE STRAIN VARIATION IN IMMUNE RESPONSE TO MYCOBACTERIUM LEPRAE

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In order to investigate the genetic control of the murine immune response to Mycobacterium leprae (M.leprae) cellular and humoral responses following intradermal immunisation with irradiated M.leprae have been compared in nine inbred strains of mice.

On the basis of in vitro lymphocyte transformation tests (LTT) using sensitized lymph node lymphocytes it has been possible to classify mouse strains into high (eg B10.M) and low (eg BALB/c) responder groups. A similar classification was obtained when delayed type hypersensitivity and lymphokine responses were compared in the various strains. In addition, limiting dilution analysis of the frequency of M.leprae reactive T cells generated by immunisation was carried out and showed a general correlation with the results of the other studies. In contrast to the cell mediated responses, anti-mycobacterial antibody production as assessed by ELISA showed an inverse relationship between antibody titre and T cell proliferative response for a given strain. We have also investigated the susceptibility of high and low responder strains to M.leprae infection, and to the effects of vaccination with killed M.leprae.

109. THE INFLUENCE OF ANTI-MYCOBACTERIAL CHEMOTHERAPY ON DELAYED HYPERSENSITIVITY REACTIONS (DHR) IN LEPROSY PATIENTS

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Skin tests using PPD and Rees skin test antigen (RSTA), a soluble extract of Mycobacterium leprae, were performed in 53 treated leprosy patients, 52 newly diagnosed untreated

leprosy patients and 78 household contacts of untreated leprosy patients in northern Bangladesh. In addition a small group of 20 leprosy hospital workers and a further group of 50 indigenous subjects with no known exposure to leprosy were studied.

Untreated patients showed significantly fewer positive PPD and RSTA skin test reactions than treated patients. Thus treatment of leprosy patients apparently enhances their ability to produce a delayed hypersensitivity reaction to mycobacterial antigens. This underlines the necessity for study of untreated patients in immunological studies of leprosy, since treatment alters immune status and misleading results may be obtained if treated patients are studied alone. Despite apparently strong cell-mediated immunity (CMI) to Mycobacterium leprae in their lesions, a high proportion (48.5%) of the untreated borderline-tuberculoid patients did not react to RSTA. Many of these patients would down-grade on the Ridley-Jopling scale without treatment. This phenomenon may be associated with the general depression of CMI which was shown in this study by the failure of 39.4% of these patients to respond to PPD. Consistent with this explanation, the percentage of borderline-tuberculoid patients with positive skin tests to RSTA increased significantly with treatment, while the area of induration amongst the responders is not significantly different. Thus specific delayed hypersensitivity to M.leprae appears to be similar between the treated and untreated groups, the difference reflecting general suppression of delayed hypersensitivity to mycobacteria in untreated patients. This contrasts with the lepromatous patients in whom the percentage of responders and the size of the responses increased following treatment, perhaps reflecting the development of specific immunity to M.leprae and increased general CMI.

110. EFFECT OF RECOMBINANT INTERLEUKIN 2 ON T CELL UNRESPONSIVENESS TO MYCOBACTERIA IN ADVANCED DISSEMINATED TUBERCULOSIS

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Peripheral blood mononuclear cells (PBMC) from patients with a localized form of pulmonary tuberculosis (Loc-TB) proliferate and release interleukin 2 and immune interferon when exposed to PPD in vitro. In contrast, PBMC from patients with advanced disseminated tuberculosis (Dis-TB) do not repond to PPD. A limiting dilution analysis revealed a lower frequency of PPD reactive precursor cells in cultures of Dis-TB PBMC as compared to cultures from Loc-TB patients. Moreover, a lower percentage of PBMC expressing interleukin

2 (IL-2) receptor has been found in Dis-TB patients than in Loc-TB patients. We also investigated the influence of exogenous IL-2 on both responder and non-responder cultures. Human recombinant IL-2 augmented both cell proliferation and interferon production in the low responder cultures, suggesting that IL-2 could be used for the treament of the immunological anergy present in Dis-TB patients.

111. IMMUNOHISTOCHEMICAL STUDY OF SJOGREN'S SYNDROME

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We have studied labial biopsies from 7 patients with primary and 9 patients with secondary Sjogren's syndrome. Using monoclonal antibodies, HLA class I and class II antigens; T lymphocytes; alpha, ß and gamma interferons; IL-2 and the IL-2 receptor (TAC) were identified. The major features, common to both types of Sjogren's were the frequent identification of HLA class I antigens in the salivary ducts, acini and infiltrating lymphocytes (many of which were T cells bearing HLA class II antigens) and the paucity of alpha and ß interferons, IL-2 and TAC. Whilst gamma interferon was apparently detected in the acini and ducts of most biopsies the strongly positive appearance of some individual duct cells raises the possibility of a cross-reacting antigen. The results thus show major differences with experience in polymysitis where HLA class I expression was closely matched to that of the interferons.

112. THYMIC AUTOANTIBODIES IN ARC & AIDS PATIENTS

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There is a yet unknown relationship between the neuro-endocrine portion of the thymus and retroviral proteins: firstly, thymic epithelial cells react with monoclonal antibodies to p19 (HTLV-1), p15E (Mu and FeLV), p18 (HIV) and gp41 (HIV); secondly, thymosine-alpha$_1$ shows a structural homology with p17 from HIV. An effective antibody response to retroviral protein after HIV-infection in an attempt to neutralize the virus could hence result in an autoimmune response to thymus epithelial cells or thymic hormones. That this may indeed be the case is suggested by post-mortem reports on the presence of an autoimmune assault on the thymus in children and adults who have died of AIDS. We have looked into the presence of thymic autoantibodies in the serum of HIV-infected homosexuals, ARC and AIDS patients using an indirect IFL technique on rat thymus. It appeared that 50-60% of ARC and AIDS patients had high titres of such antibodies (exceeding

1:40, and predominantly of IgG-class). The cells reactive with the antibody were also positive for p15E and thymic-hormones. Normal healthy individuals, asymptomatic HIV-infected homosexuals and patients with thyroid autoimmune disease and SLE were negative or showed low titres of the antibodies (<1:20).

Whether thymic antibodies play a role in the destruction of the gland and the impairment of cell mediated immunity in ARC and AIDS needs further clarification; it is relevant to note that a similar type of antibody has been described in the diabetes-prone BBW-rat, which also shows severe impairment of cell mediated immunity, low numbers of Th-cells and thymic atrophy.

113. IgM SPECKLED ANTI-NUCLEAR ANTIBODY IN CULTURE SUPERNATANTS OF TRANSFORMED BLOOD MONONUCLEAR CELLS

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Some culture supernatants of Epstein-Barr virus (EBV) transformed pokeweed mitogen (PWM), and P.falciparum culture supernatant stimulated blood mononuclear cells from rheumatoids and normal subjects, produced speckled nuclear staining with anti-human IgM-FITC on monolayers of cultured cells. Nuclear staining was abolished in 5/7 EBV-transformed culture supernatants, 2/2 PWM, and 2/3 P.falciparum stimulated samples which were absorbed with heat-aggregated human IgG but not in controls. Cells from one of the EBV-transformed positive cultures with lambda light chain specificity tested showed reactivity with rabbit IgG.

114. IMMUNOGLOBULINS STIMULATING & BLOCKING ADRENAL GROWTH

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The hyperthyroidism of Graves' disease is nowadays considered to be due to immunoglobulins (Ig) stimulating thyroid hormone-production. At an earlier occasion we have reported on the presence of Ig's capable of stimulating thyroid growth; these Ig's occur in goitrous Graves' disease and simple goiter. Ig's blocking the effect of TSH have also been described, particularly in thyroid atrophy. The present report concerns a study on Ig's affecting the growth and hormone-production of the adrenal.

Methodology: Guinea pig adrenal segments were kept in organ culture (5 hrs, 37°C) with $ACTH_{1-39}$, $ACTH_{1-13}$, $ACTH_{1-24}$ or $ACTH_{18-39}$ added. At the end of the culture period segments were snap-frozen. Cell growth was determined by nucleic acid cytophotometry, whereas steroidogenesis was measured quantitatively by means of cytophotometry of ascorbate depletion and RIA of the in vitro produced cortisol. Ig's of 2 patients with micronodular adrenocortical dysplasia were tested in the system, as were Ig's of 18 Addison patients.

Results: $ACTH_{1-39}$ was found to stimulate both cell growth and steroidogenesis (optimal dosages 0.1-1.0 µu per ml medium), whereas $ACTH_{18-39}$ predominantly stimulated adrenal cell growth. In contrast, $ACTH_{1-24}$ had a stronger effect on steroidogenesis. The Ig's of the 2 patients with the adrenal dysplasia stimulated both adrenal cell growth (optimal at 30-60 µg per ml) and ascorbate depletion (optimal at 125-180 µg per ml). Fourteen of the 18 Ig's of Addison patients were capable of inhibiting $ACTH_{1-39}$ induced adrenal cell growth. As controls, Addison's disease due to tuberculous destruction and hypopituitarism were taken, as were sera from patients with Cushing's disease and healthy controls; all were negative. These preliminary data strongly suggest a role of (receptor?) antibodies in the pathogenesis of some forms of Cushing's syndrome and Addison's disease.

115. ACETYLCHOLINE RECEPTOR (AChR) & ANTI-AChR ANTIBODY SPECIFICITIES IN THE THYMUS IN MYASTHENIA GRAVIS

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Monoclonal anti-human AChR antibodies have been used to investigate, by competion, the binding specificities of anti-AChR in 67 myasthenia gravis (MG) patients, and to locate AChRs on muscle-like (myoid) cells in MG and control thymus.

Specificities differed between patients but in individuals were similar in serum and thymus culture supernatants (n=10), and did not change significantly after thymectomy (n=22), in spite of an overall fall in anti-AChR.

All monoclonal anti-AChR bound to myosin$^+$ or troponin$^+$ cells in the thymic medulla; these cells did not differ quantitatively or qualitatively between 21 patients and 14 controls. Myoid cells in MG patients did not appear to be the focus of an immunological attack.

116. A MINORITY OF PATIENTS WITH RHEUMATOID ARTHRITIS SHOW A DOMINANT REARRANGEMENT OF T CELL RECEPTOR ß CHAIN GENES IN SYNOVIAL LYMPHOCYTES

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The clonality of T lymphocytes isolated from the synovial fluid and peripheral blood of patients with rheumatoid arthritis was investigated by restriction enzyme fragment mapping of the rearrangements of the ß chain gene of the T cell antigen receptor. Three patients showed a dominant rearrangement amongst their synovial fluid T cells which was not seen in their peripheral blood T cell population suggesting the presence of a predominantly T cell clone. However eight of eleven patients examined demonstrated polyclonal T cell populations in both their synovial fluid and peripheral blood. Of four synovial fluid T cell lines investigated one showed evidence for a dominant T cell clone. The possibility that the predominating clones are directed against auto-antigens present in the joint will be discussed.

117. AUTO-ANTI-IDIOTYPES IN SLE

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Most studies of auto-anti-idiotypes in lupus sera have detected such antibodies using antigen inhibition systems. We have devised an idiotype inhibition assay to look for serum antibody activity against a public idiotype, $IdLL^a$. This determinant is present on a wide range of human monoclonal antibodies of different ligand-binding specificities, and is highly immunogenic in experimental animals.

Immunoglobulin (Ig) preparations from nine patients with systemic lupus erythematosus and 33 healthy donors were absorbed on normal Ig, and then passed over a column bearing a human monoclonal antibody which carries the $IdLL^a$ idiotope. An eluate was obtained from the Ig fraction of one lupus patient only. This eluate inhibited the binding of monoclonal anti-$IdLL^a$ to $IdLL^a$.

This result demonstrates the presence in this patient's serum of auto-anti-idiotypic antibodies directed against a recurrent idiotope. The fact that $IdLL^a$ itself was not detectable in the patient's whole Ig fraction suggests that such auto-anti-idiotypic antibodies may play a role in

immunoregulation in this individual. Our data indicate, however, that naturally-occurring antibodies against this particular idiotope are rarely detectable in the sera of lupus patients and normal subjects.

118. **EPITOPES & RNA BINDING SITES ON LA/SS-B NUCLEAR ANTIGEN**

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Monoclonal anti-La antibody SW5 was used to affinity purify rabbit human La from thymus and placental villi respectively. Digestion of the antigens with various proteases singly or in combination produced immunoreactive polypeptides with molecular weights down to 10 kD. This polypeptide bound three monoclonal anti-La antibodies and the reference human anti-La sera, suggesting that the La epitopes are on a restricted 10 kD domain. Since one of these monoclonal antibodies binds at or near the major La RNA binding site, these results suggest that the immuno-reactive 10 kD domain may be associated with the RNA binding site.

119. **HUMAN MONOCLONAL ANTIBODIES TO POLY (ADP-RIBOSE) CROSS REACT WITH DNA**

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Poly (ADP-ribose) is a breakdown product of NAD and thought to be concerned with DNA synthesis and repair. We have studied the antigen binding profiles of 5 human hybridoma derived monoclonal antibodies from 2 patients with leprosy, and 1, designated 134, from a lupus patient which bind to poly (ADP-ribose). In direct binding ELISA assays these monoclonal antibodies also bind strongly to ssDNA and more weakly to dsDNA. In competition assays up to 70% of the binding to poly (ADP-ribose) is inhibited by ss DNA suggesting that there may be sharing of some conformational determinants. However, idiotypic analysis with R 134 has not shown sharing of the 134 idiotype.

120. **MONOCLONAL ANTIBODIES RAISED BY IMMUNISATION WITH GROUP A STREPTOCOCCAL CELL WALLS WILL BIND TO THE IMMUNOGLOBULIN OF RHEUMATOID ARTHRITIS PATIENTS**

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It has been reported that some patients with Rheumatoid arthritis have raised levels of antibody to the polysaccharide/petidoglycan complex of the cell walls of Group A streptococci (Johnston et al. Clin. exp. Immunol. 55, 115, and 61, 373), and that mice immunised with affinity column purified rheumatoid factor also develop antibody to this streptococcal preparation. A major determinant on Group A streptococci is N-acetyl glucosamine (GlcNAc) linked to polyrhamnose. The Oxford Oligosaccharide Group have shown that the N-linked sugars on the Fc of IgG from RA patients terminate with GlcNAc significantly more frequently than is normal (Parkeh et al., (1985) Nature, 316, 452). We hypothesised therefore that the cross-reactive epitope between rheumatoid Ig and Group A streptococci might be the GlcNAc. We have found that monoclonals to GlcNAc can be raised by immunisation with this organism and that some of these will bind to the abnormally glycosylated Ig preparations. Thus there is a simple explanation for the findings with Group A streptococcal walls, which does not require the concept of idiotype complementarity.

121. **ANTIBODY AFFINITY AND IgG SUBCLASS OF RESPONSES TO TETANUS TOXOID IN PATIENTS WITH SLE & RA**

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Antibody affinity, measured by a radioimmunoprecipitation assay, and the IgG subclass of antibody, measured by ELISA using monoclonal antibodies, have been assessed in 24 patients with SLE, 29 patients with RA and in 33 age and sex matched controls before and after immunization with tetanus toxoid. All the groups showed a similar increase in the amount of antibody produced after immunization but significant differences were found in antibody affinity. SLE patients had a wide range of responses with some patients producing antibody of very high affinity whereas RA patients had signficantly lower affinity antibody and failed to show affinity maturation. In addition, differences were found in the IgG subclass distribution of the antibody responses. Controls had a predominantly IgG1/IgG4 response before and after immunization but SLE and RA patients showed

marked restriction to IgG1 antibody before immunization and a more general response to all the subclasses after immunization. A significant association between the production of IgG4 antibodies and low affinity responses was seen in the controls but not in the patients with SLE or RA.

122. **QUANTITATION OF PLATELET BOUND C3d AND SERUM C3d LEVELS IN PATIENTS WITH IMMUNE THROMBOCYTOPENIC PURPURA (ITP)**

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The detection of complement binding antibodies in ITP patients is important in predicting the response to currently available treatments for the disease. Recent attempts to identify components of complement activation C3c and C3d both bound to the platelet and free in the serum of such patients have resulted in conflicting reports. To examine this problem we quantified the amount of IgG, IgM, C3c and C3d bound to the platelet surface by flow cytometry and measured the plasma C3d levels by rate nephelometry in ten ITP patients. Good correlation (r=0.850) was found between the levels of platelet associated IgM (PAIgM) and C3d and also between PAIgM and plasma C3d (r=0.919). Correlation was also found between the patients platelet count, and plasma C3d (r=-0.914).

123. **INTRAVENOUS IMMUNOGLOBULIN TREATMENT OF RECURRENT AND CONTINUOUS ERYTHEMA MULTIFORME**

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The clinical and immunological response was investigated in six patients with recurrent and continuous Erythema Multiforme receiving high dose intravenous immunoglobulin (IVIgG). All patients showed regression of their lesions and one had a sustained clinical remission. Abnormalities of cellular and humoral immunity were demonstrated prior to treatment, including; deficiencies in NK cells; reversed T4:T8 ratio; raised levels of circulating immune complexes and consistently low CH50 levels throughout the group. These abnormalities were all modified following IVIgG therapy. Our results suggest that Erythema Multiforme is not soley immune complex mediated and that IVIgG modifies the clinical course of the disease although its exact mechanism of action remains unclear.

124. **PRODUCTION OF IMMUNOREGULATORY mRNA SPECIES WITHIN THE RHEUMATOID JOINT**

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We have used a number of recently developed gene probes to investigate the production of IL-1 (alpha & B), IL-2, gamma-interferon PDGF and TNF mRNAs by immunocompetent cells within the rheumatoid joint. We report here for the first time that TNF and PDGF are produced in the joint. We can also confirm that IL-2, gamma-interferon and large amounts of IL-1 mRNA can be found in the cells from the synovial fluid and membranes of RA patients.

Our findings are an attempt to define which immunoregulatory mediators are present within the rheumatoid lesion and indicate possible positive feedback mechanisms which may contribute to its pathological nature.

125. **RHEUMATOID ARTHRITIS (RA) T LYMPHOCYTES EXPRESS SUBNORMAL AMOUNTS OF HLA-DR ANTIGEN AFTER PHYTOHAEMAGGLUTININ (PHA) STIMULATION**

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Resting T lymphocytes do not synthesise or express HLA-DR antigens but following stimulation with antigens or mitogens, such as PHA, they synthesise and express HLA-DR on their cell membranes. Such activated, DR+ T cells may be of importance in immune reactions since they can activate autologous/allogeneic T cells in the mixed T lymphocyte reaction (MLR). Separated peripheral blood mononuclear cells from RA patients and controls were stimulated with 2 µgs/ml PHA for 72 hrs and the proporiton of T cells expressing DR and its intensity of fluorescence were assessed by double-label immunofluorescence using DR-specific (FDRR2) and pan T cell specific (Leu 1) markers. The percent DR+ T cells in RA was not signicantly different from the controls (Figure 1) but the fluorescence intensity was significantly less (Figure 2). Thus, RA T cells appear to express less DR on their cell surface after PHA stimulation. This may be a mechanism whereby DR-related responses, such as the autologous AMLR, is reduced in RA.

126. PERGASTRICALLY-ADMINISTERED GLUTARALDEHYDE CROSS-LINKED TYPE II COLLAGEN REDUCES THE INCIDENCE OF COLLAGEN-INDUCED ARTHRITIS IN RATS

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Arthritogenic bovine type II collagen (CII) was polymerized by cross-linking with glutaraldehyde. Inbred Wistar rats were pergastrically-dosed with polymerized CII (POL CII); a form of CII which when administered intradermally in Freund's Incomplete Adjuvant (FIA) lower the incidence of CII-inducable disease. Pergastrically administered "particulate" POL CII was found to render animals resistant to arthritis on subsequent intradermal challenge with an arthritogenic dose of soluble CII in FIA. The polymerization of arthritogenic CII alters it to a collagen molecule capable of eliciting a non-arthritic response. This study demonstrates that pergastrically-administered POL CII lowers disease incidence more effectively than pergastrically-administered soluble native CII.

127. PREFERENTIAL ACTIVATION OF CYTOTOXIC-SUPPRESSOR HLA-DR POSITIVE T LYMPHOCYTES IN CHRONIC HEPATITIS B VIRUS (HBV) INFECTION IN CHILDHOOD

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Children with autoimmune chronic active hepatitis (aCAH) have preferential activation of T lymphocytes expressing Interleukin-2 receptor (IL2r) and helper/inducer phenotype. To assess whether a similar pattern of activation is present in HBV related chronic liver disease (CLD) we studied 32 children with chronic HBV infection, 24 having biochemical and/or histological evidence of CLD. HLA-DR and IL2r expression on purified T lymphocytes was assessed by direct immunofluorescence with phycoerythrin conjugated monoclonal antibodies. Functional subsets of activated T cells were studied by double staining with fluorescein conjugated anti cytotoxic/suppressor (Leu2) or helper/inducer (Leu3) antibodies. HLA-Dr positive T cells were significantly increased both in patients with (mean $\pm$ SD 14.2 $\pm$ 5.7%: $p<0.01$) and without evidence of CLD (7.9 $\pm$ 2.4%: $p<0.01$) when compared to 20 healthy children (2.8 $\pm$ 1.0%). 56% of the HLA-DR positive cells were cytotoxic/suppressor and 32% helper/inducer. IL2r positive T cells were increased in patients with CLD (2.2 $\pm$ 1.6%) but not in those without CLD (0.8 $\pm$ 0.6%) when compared to controls :(0.2 $\pm$ 0.1%). In contrast to aCAH, in chronic HBV infection there is

preferential activation of T lymphocytes expressing HLA-DR and cytotoxic/suppressor phenotype. These cells may represent the effectors of the in vitro T cell mediated cytotoxicity to autologous hepatocytes found in HBV related but not autoimmune CLD.

128. EVIDENCE OF IMPAIRED ANTIGEN NON-SPECIFIC BUT NORMAL ANTIGEN SPECIFIC SUPPRESSOR FUNCTION IN CHILDREN WITH AUTOIMMUNE CHRONIC ACTIVE HEPATITIS (aCAH)

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Children with aCAH have impaired Concanavalin A (Con A) induced suppression of Pokeweed mitogen stimulated immunoglobulin synthesis. Since this system tests non-specific immunoglobulin production, we investigated suppression of in vitro production of antibody to Tetanus toxoid (TT), a T-dependent antigen, by lymphocytes from 11 children with aCAH and 14 age-matched healthy controls, all previously immunised. Both patients and controls had similar serum anti-TT levels (mean $\pm$ SEM 1.8 $\pm$ 0.85 $x10^{-3}$ IU/ml, 2.0 $\pm$ 0.86 $x10^{-3}$ IU/ml) and in vitro production of anti-TT in response to optimal dose of TT (0.005 IU/ml) (5.3 $\pm$ 0.88 $x10^{-3}$ IU/ml, 6.7 $\pm$ 0.94 $x10^{-3}$ IU/ml) as measured by ELISA. Suppressor function was induced by 24 hour incubation of lymphocytes with high dose TT (5IU/ml) or with 20 µg/ml Con A. Washed cells were then added to stimulated autologous lymphocytes. TT-induced suppression of anti-TT production was similar in patients (69.8 $\pm$ 4.2%) and in controls (72 $\pm$ 3.8%). In contrast, Con A induced suppression of anti-TT was significantly lower in patients (15.7 $\pm$ 2.5%) than in controls (46.7 $\pm$ 4.9% $p<0.01$). Our data indicate that antibody production to a T-cell-dependent antigen is under the control of at least two regulatory mechanisms, one antigen specific and one antigen non-specific, only the latter being defective in aCAH.

129. FUNCTIONAL ANALYSIS OF LESIONAL T LYMPHOCYTES

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The presence of disease-specific T cells in inflammatory infiltrates of patients with nickel-contact dermatitis was investigated by direct cloning of the T cells with a 100% cloning efficiency. In three experiments 7-15% of the $CD4^+8^-$ clones were shown to be specific for nickel in a

proliferation assay. The frequency of nickel-specific clones did not vary considerably in acute versus chronic lesions.

The crucial role of antigen presenting cell heterogeneity in these experiments was stressed by the fact that 5 out of 8 nickel-specific clones exclusively recognized nickel when presented by skin-specific Langerhans cells.

130. **EFFECT OF GLUCOCORTICOIDS ON LYMPHOKINE-INDUCED INHIBITION OF MYCOBACTERIUM TUBERCULOSIS BY MURINE AND HUMAN MACROPHAGES; FURTHER EVIDENCE FOR A "MAF" WHICH IS NOT GAMMA INTERFERON**

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Recombinant murine gamma interferon (IFN-gamma) induces total inhibition of growth of virulent M.tuberculosis in murine peritoneal macrophages, but it induces no inhibition of M.tuberculosis in the presence of dexamethasone (10^{-8}M), or of corticosterone or cortisol at physiological concentrations. However these steroids do not block activation by IFN-gamma of the oxygen reduction pathway, which is therefore unlikely to be involved in the stasis of M.tuberculosis.

Supernatants from some mycobacterium-responsive murine T cell clones differ from recombinant IFN-gamma in that although they also induce total inhibition of M.tuberculosis by the murine macrophages, their effect is not diminished by steroids. This is further evidence for the existence of a second MAF.

Human monocyte-derived macrophages develop much weaker but measure ability to inhibit M.tuberculosis in the presence of recombinant human IFN-gamma, in spite of massive activation of the oxygen reduced pathway. This weak IFN-gamma-induced effect differs from the powerful effect seen with murine peritoneal macrophages in that it is totally unaffected by steroids. Preliminary evidence suggests that a steroid-sensitive anti- mycobacterial mechanism does exist in human cells, but that in contrast to the situation in the mouse, IFN-gamma is not the lymphokine which activates it.

131. **INTRACELLULAR CALCIUM CHANGES IN STIMULATED MACROPHAGES**

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The activation of macrophages probably involves intracellular calcium since calcium ionophores can substitute for several activation signals. We have used calcium sensitive fluorescent dyes to measure intracellular calcium in macrophage cell lines.

Our data show clear changes occurring within minutes in intracellular free calcium concentrations in response to several stimuli including calcium ionophores, platelet activating factor, chemotactic peptide and ATP. However other stimuli, lipiopolysaccharide and interferon gamma, known to activate macrophages, do not appear to cause similar changes.

132. **MECHANISMS INVOLVED IN THE PHAGOCYTOSIS OF CANDIDA GUILLERMONDII BY HUMAN MONOCYTES AND ALVEOLAR MACROPHAGES**

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We have studied opsonin-dependent phagocytosis of C.guillermondii by human monocytes and Alveolar Macrophages (AM). The role of the Complement Receptors CR1 and CR3 on this cell function was evaluated by blocking the receptors with monoclonal antibodies prior to assay. Under these conditions we observed partial inhibition of phagocytosis. Blockade of the mannose-fucose and b-glucan receptors was also performed using Mannan and b-glucan, and inhibitory effects on phagocytosis were observed. Complete inhibition of phagocytosis was however still not achieved when mannan and/or b-glucan blockade was undertaken together with CR1 and CR3 blockade.

These results suggest that whilst phagocytosis of C.guillermondii by human monocytes and AM is partially dependent on complement, mannose-fucose and b-glucan receptors, unidentified receptors may also be involved in this cell activity.

133. **GP41-LIKE FACTORS CIRCULATE IN HIV-INFECTED HOMOSEXUALS AND AIDS PATIENTS AND INFLUENCE MONOCYTE CHEMOTAXIS**

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Severe impairment of cell mediated immunity is one of the characteristics of AIDS, which disease is nowadays known to be etiologically associated with infection with the lymphotropic retrovirus HIV. An AIDS-like immunodeficiency occurs in cats and mice after infection with feline or murine leukemia retrovirus (FeLV and MuLV); and this form of immunodeficiency is thought to be due at least in part to the effect of an immunosuppressive capsular protein of MuLV en FeLV, viz. p.15E. P15E is able to suppress d.h. reactivity, to hamper IL-2 production and to inhibit monocyte chemotaxis. Immunosuppressive factors sharing a structural homology with p15E can normally be produced by mammalian cells: e.g. carcinoma cells, lymphoid cells and most noteworthy by thymic epithelial cells. Since there exists a limited structural homology between p15E and the capsular protein gp41 of HIV, we have studied the effect of gp41 (produced by recombinant techniques) on a function of cell mediated immunity, i.e. the FMLP-induced polarization of monocytes. We have also looked for the presence of gp41 like factors in the circulation of groups of homosexuals in a metropolitan surrounding before and after HIV-infection, and in ARC and AIDS patients. Recombinant gp41 was able to inhibit FMLP induced monocyte polarization, this effect was eleviated by two monoclonal antibodies to gp41, but not by an antibody to p15E. The sera of non HIV-infected homosexuals contained factors which were able to inhibit monocyte polarization, but these were of low molecular weight (<than 25kD) and could be absorbed by an alpha p15E; they may represent an endogenously produced immuno-suppressive factor. Interestingly such factors gradually disappeared from the circulation after HIV-infection; they were replaced by larger inhibitory factors (between 25 and 50kD) reactive to monoclonals specific for gp41. In AIDS-related syndrome levels of these gp41-like factors dropped - probably due to the effect of naturally occurring alpha-gp41 in such patients - to clearly rise again in AIDS. Our data may suggest a role in HIV-related immunodeficiency for retroviral peptides mimicking naturally occurring immunosuppressive endocrine factors.

134. **IN-VITRO STUDY OF THE EFFECTS OF INTRAVENOUS IMMUNOGLOBULIN THERAPY ON THE OPSONISING CAPACITY OF SERA FROM PATIENTS UNDERGOING BONE MARROW TRANSPLANTATION**

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Patients undergoing bone marrow transplantation (BMT) have an increased susceptibility to serious bacterial and fungal infections. Significant risk factors include the state of neutropenia, graft-versus-host disease and concomitant immunosuppressive therapy. Hypogammaglobulinaemia as well as IgG subclass deficiencies may also have an important contributory role to play in intercurrent infection. As part of a clinical study undertaken in 60 patients to evaluate an intravenous immunoglobulin preparation for prevention of infection after BMT, sequential serum samples were taken from recipients of IgG therapy and from controls who received a placebo. Opsonisation studies were performed using chemiliminescence in which the antigen challenge was with organisms recovered from septicaemic episodes in BMT patients and included Pseudomonas aeruginosa, Staphylococcus epidermidis and Candida albicans. In each experiment an organism was opsonised with serum and added to neutrophil preparation that had been obtained from a healthy volunteer. The results of these experiments will be presented and correlated wiht the findings of the clinical study.

135. **QUANTITATIVE COMPARISON OF CLASS II MHC POSITIVE CELLS & MICROGLIAL/MACROPHAGE CELL TYPES IN WHITE MATTER FROM CONTROL & MULTIPLE SCLEROSIS BRAIN TISSUE**

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The immunocytochemical characteristics of potential antigen presenting cells in the brain has been investigated using monoclonal antibodies against class II MHC and a pan macrophage marker which stains cells of microglial morphology. Titration of these antibodies on frozen sections of control and MS brain demonstrates an increase in the number of microglial/macrophage-like cells in apparently normal MS white matter. The regular arrangement of these cells and the degree of correlation with MHC class II staining may give a measure of immune reactivity in MS tissue.

136. EFFECT OF EXOGENEOUS PROSTAGLANDIN E2 & INDOMETHACIN ON THE HLA/DR ANTIGEN EXPRESSION OF PERIPHERAL BLOOD MONOCYTES IN CULTURE

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Monocytes are known to mediate immunosuppression by releasing soluble mediators such as prostaglandins. We investigated the effect of exogeneous PGE2 on the HLA/DR antigen expression on monocytes in culture. Monocytes from 8 healthy controls were purified by plastic adherence (>92% MO2 positivity) and cultured at 4 x 10^5 cells/well in RPMI medium supplemented with 10% FCS. PGE2 and indomethacin were added to these cultures and the HLA/DR antigen levels on the monocytes measured by a modified radioimmunoassay and counted in a gamma-counter on day 7.

PGE2 (range 10^{-5}M to 10^{-12}M) does have a stimulatory effect on HLA/DR antigen expression of 7 day cultured monocytes, but this is more evident when low concentrations are used. Indomethacin can reverse the inhibitory effect of high concentrations of PGE2. Indomethacin, even at low concentration (0.1 ug/ml) can enhance HLA/DR expression on monocytes. These findings suggest a further role for anti-PGE2 therapy in disease states such as rheumatoid arthritis where we have shown reduced surface HLA/DR antigen expression on monocytes (1).

(1) Hassan et al (1986) Brit. J. Rheum. 25, 102.

137. EXPRESSION AND MODULATION OF MHC CLASS I AND II PRODUCTS ON HUMAN PARATHYROID CELL MONOLAYERS

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There is increasing evidence that MHC molecules play an important role in tumour immunosurveillance.

MHC Class I and II molecule expression was studied by IFL on parathyroid cell monolayers obtained from adenoma operations. Class I molecules were consistently expressed both on the cell surface and weakly in the cytoplasm in almost 100% of the parathyroid cells. In 6/15 parathyroid specimens, a proportion (5-15%) of epithelial cells also showed a spontaneous expression of Class II molecules.

Increased expression of Class I Ags after incubation with r-IFN alpha, ß, gamma, was observed. DR induction was obtained by r-IFN gamma, and in 2/15 preparations also by r-IFNß.

HLA-DP, DQ product expression was induced by r-IFN-gamma (500U/ml) only on 4/15 and 5/15 cases respectively.

PHA, IBMX, EGF were not able to modulate MHC expression.

Conclusions: A proportion of cultured epithelial cells from parathyroid adenomas express HLA D/DR products which can be modulated by Interferons.

138. CLASS II EXPRESSION BY HUMAN THYROCYTES AFTER SV40 VIRUS TRANSFECTION

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Human thyroid cells were transfected with a defective SV40 virus and a cell population with extended life span was obtained. These cells were shown to be epithelial by IFL staining with anticytokeratin monoclonal antibodies and by electron microscopy. 5 to 10% of these cells spontaneously expressed Class II molecules on their surface and in their cytoplasm as detected by IFL, whereas no Class II expression was found in the parental non-transfected cells. 30 clones have been obtained; some individual clones retain a fixed percentage of Class II positive cells (10 to 50%) and others are completely devoid of them. These characteristics have constantly been found during a prolonged period of culture.

Conclusion: Viral transfection with SV40 virus can induce inappropriate Class II expression on human thyrocytes. Endocrine cell lines with different proportions of Class II positive cells could be relevant to the study of thyroid autoimmunity.

139. IN VITRO INDUCTION OF HLA CLASS II EXPRESSION IN HUMAN BETA CELLS: REQUIREMENT FOR A TWO MEDIATOR SIGNAL

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Inappropriate HLA Class II expression by pancreatic beta cells has been detected in type I diabetes but attempts to induce Class II in vitro in human beta cells have so far been unsuccessful. We have tested rIFN-gamma, -alpha and -beta, rIL-1, rIL-2, Tumor Necrosis Factor (TNF) and Purified Lymphotoxin (LT) either alone or in different combinations on human pancreatic monolayer cell cultures. HLA expression was assessed by immunofluorescence using MoAbs to Class II molecules. Beta cells were identified by

simultaneous staining with MoAbs to human C-peptide by double immunofluorescence. None of the lymphokines was able to induce Class II expression in beta cells when tested separately. However TNF and LT, when used in conjunction with IFN-gamma at doses ranging from 10 to 1.000 Units/ml, were able to induce Class II expression in human pancreatic beta cells. This Class II expression induced in vitro was detectable both in the membrane of viable beta cells and in their cytoplasm after fixation and also by the use of MoAb VIC-YI that reacts exclusively with the cytoplasmic invariant chain of Class II molecules.

140. INDUCTION OF CLASS II MHC ANTIGENS IN VITRO ON ISLETS ISOLATED FROM BB/E RATS

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We have previously shown that aberrant expression of class II MHC antigens occurs on pancreatic ß cells in the BB/E rat and this has also been described in human IDDM. Neither the significance of this finding nor the mechanism by which it occurs is understood. Recombinant Interferon - gamma (r. IFN gamma) has been shown to induce class II MHC antigen on both rat and human thyroid epithelial cells in vitro but not on normal rodent or human pancreatic ß cells. Susceptibility to autoimmune disease is linked to the MHC complex. The ability of r IFN gamma to induce class II expression in vitro on pancreatic islet ß cells has therefore been investigated by exposing islets isolated from BB/E and normal Wistar rats to r IFN gamma and then staining dispersed islet cells successively with monoclonal antibodies specific for rat class II MHC antigens and insulin. Induction of class II expression was never observed on islet cells obtained from either normal Wistar rats or rats from the BB/E low diabetes incidence (<2%) subline. In contrast islet cells from rats from the BB/E high incidence (60-70%) subline expressed class II antigen following culture with r IFN gamma. Findings suggest that the ability or r IFN gamma to induce class II expression depends on genotype. The mechanism of this effect may be via previous cytokine-mediated target cell damage.

141. SUPPRESSION OF RENAL ALLOGRAFT REJECTION IN THE RAT USING PROTEIN MICELLES CONTAINING CLASS I MHC ANTIGEN

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We have shown that pretreatment of DA ($RT1^a$) recipients with Lewis ($RT1^l$) liver cells expressing MHC class I antigen only produce indefinite survival of a subsequent Lew renal allograft, whereas pretreatment with soluble class I antigen alone is ineffective. In this study pretreatment of DA rats with protein micelles containing LEW class I antigen, (i.v), 7 days before transplantation, they accepted their grafts indefinitely, (MST >100d). These animals required no further form of immunosuppressive therapy.

Protein micelles were prepared by desalting of deoxycholate solubilised, affinity purified class I antigen, (isolated from liver). The dose of protein micelles used was derived by a comparative absorption assay. An equivalent amount of class I was given as was present on an effective number of viable liver cells ($5x10^7$ cells as above).

This effect was donor-specific as DA recipients that received an equivalent amount of class I antigen purified from WAG ($RT1^u$) liver was not effective and these animals rejected their grafts in the same time as untreated controls, (MST 10d).

142. INTERLEUKIN-2 RECEPTOR ANTIBODY THERAPY IN RAT CARDIAC AND RENAL ALLOGRAFT MODELS

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Monoclonal antibodies to the interleukin-2 receptor (IL-2R) may offer a means of specific immunosuppression as they are directed against immunologically activated but not resting lymphocytes. Some success has been achieved with IL-2R antibody treatment in murine cardiac allograft models [1,2]. We compared the effect of an anti-rat IL-2R antibody, ART 18, in two different organ transplantation models.

ART 18 was administered intravenously to DA-$RT1^a$ recipients of LEW-$RT1^l$ heterotopic heart or orthotopic kidney allografts at a dose of 300 ugs/kg/day for 10 consecutive days post-transplantation. IL-2R targeted therapy prolonged the survival of cardiac allografts, in some cases indefinitely. However, the same regimen was ineffectual in prolonging the survival of renal allografts

(MST 10 days vs 10.3 days in untreated controls). Interestingly, combining ART18 with a sub-therapeutic dose of Cyclosporin A (1.5mg/kg/day orally for 10 days post-transplantation - which is never effective on its own) prolonged the survival time after kidney transplantation in 50% of animals, in some cases indefinitely. Such combination therapy has been shown to be highly effective in rat heart transplants [3].

Thus, rat cardiac and renal allografts differ greatly in their ability to be enhanced by IL-2R antibody therapy. ART 18 acts syngergistically with Cyclosporin A, an observation with important potential clinical implications.

References

1. Kirkman RL et al. Transplantation 1985; 40: 719-722.
2. Kupiec-Weglinski JW et al. Proc Natl Acad Sci USA 1986; 83:-2624-2627
3. Diamantstein T, Volk KD, Tilney NL, Kupiec-Weglinski JW. Immunobiology. (In press).

143. PROLONGATION OF MURINE CARDIAC ALLOGRAFTS BY ANTI-L3T4 MONOCLONAL ANTIBODY TREATMENT

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The ability of monoclonal antibodies directed against either L3T4, Thy-1, Lyt-2 or IL2-receptor antigens to prolong the survival of fully vascularised cardiac allografts was tested in a high responder [C57.B10 to C3H] and low responder [C57.B10 to DBA2] murine strain combination. In both groups, anti-L3T4 antibody significantly delayed graft rejection [C57 to DBA MST>60 days, control MST=11 days; C57.B10 to C3H MST>35 days, control MST=13 days] while the other three antibodies had no effect. Cytofluorographic analysis revealed substantial depletion of peripheral and splenic T helper cell subpopulations [60-90%] following anti-L3T4 antibody treatment. Monoclonal anti-L3T4 antibody therapy prolongs survival of cardiac allografts in the mouse, possibly by selective depletion of T helper cell subsets.

144. SUBCLASS MAY NOT BE IMPORTANT IN THE MECHANISM OF ACTION OF MONOCLONAL ANTIBODY THERAPY IN VIVO

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We compared the effect of two interleukin-2 receptor (IL-2R) antibodies (MRC OX39 and ART 18) on graft survival in a rat heterotopic cardiac allograft model using a LEW-$RT1^1$ to DA-$RT1^a$ strain combination. Both antibodies precipitate a 55kD protein and are of the mouse IgG1 subclass. Previous studies have shown that ART 18 prolongs cardiac allograft survival and we confirmed this in our model. However, MRC OX39 (even at three times the effective dose of ART 18) was completely ineffective. Thus, two antibodies directed against the same antigen and of the same subclass have different in vivo effects in the rat. This suggests that subclass is not of absolute importance in the mechanism of action of monoclonal antibody therapy.

145. SUPPRESSION OF RAT RENAL ALLOGRAFT REJECTION BY SOLUBLE CLASS I ANTIGENS & A SINGLE LOW DOSE OF CYCLOSPORIN A

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In the Lewis ($RT1^1$) to Dark Agouti ($RT1^a$) strain combination when DA recipients were treated with an unpurified soluble preparation of Lewis class I antigens, prepared from liver, (i.v.), 7 days before transplant, they accepted their grafts indefinitely, (MST >100d), provided recipients also received one low dose of cyclosporin A on the third day after transplantation (10mg/kg). When soluble class I antigen was given alone with no cyclosporin treatment these animals rejected their grafts at the same time as untreated controls, (MST 10d). The dose of soluble antigen was quantitated by a comparative absorption assay, giving equivalent doses of class I as was present on an effective dose of viable liver cells ($5x10^7$ or above). The effect was donor-specific as recipients treated with soluble WAG ($RT1^u$) class I antigens all rejected their grafts even with cyclosporin treatment, (MST approx. 10d).

146. **PRETREATMENT WITH TRANSFECTED L-CELLS EXPRESSING DONOR CLASS I MHC ANTIGEN CAUSES SPECIFIC PROLONGATION OF MURINE CARDIAC ALLOGRAFT SURVIVAL**

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In order to investigate the effects of isolated K or D locus Class I gene products on murine cardiac allograft survival, C3H [H-2^k] recipients were pretreated with syngeneic L-cell transfectants [H-2^k] expressing either donor K or D locus molecules of the C57.B10 [H-2^b] haplotype [K^b or D^b]. Allograft survival was prolonged, but not indefinitely, in a donor-specific and dose-dependent manner. However, pretreatment with EL cells, which share minor histocompatibility antigens, in addition to both Class I K and D locus molecules with the donor organ [K^b and D^b] can induce indefinate graft survival. We are now pretreating recipients with doubly transfected L-cells expressing the K^b and D^b molecules but no donor minor histocompatibility antigens.

147. **TUMOUR INFILTRATING LYMPHOCYTES: ANALYSIS BY FINE NEEDLE ASPIRATION CYTOLOGY AND FLOW CYTOMETRY. A PILOT STUDY**

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The usefulness of fine needle aspiration cytology has been examined in an attempt to analyse the tumour infiltrating lymphocytes in colorectal tumours. The cellular aspirate from the tumour has been stained with monoclonal antibodies specific for pan T cells, helper cells, cytotoxic/ suppressor cells, natural killer cells, and B cells. Subset enumeration has been performed on a flow cytometer and the results compared with lymphocyte subsets of peripheral blood from the same patients. The aspirates from 13 tumours have been compared with 19 peripheral blood assays. In the remaining 6 cases, insufficient cells were gained from the tumour for analysis. The results were as follows:

MONOCLONAL ANTIBODY

	LEU-4	LEU-3	LEU-2	LEU-11	LEU-12	RATIO#
BLOOD	68% (41-89)	44% (23-73)	21% (6-54)	16% (4-43)	5% (2-26)	1.9 (0.4-3.8)
TUMOUR	33%* (19-73)	29% (11-49)	22% (4-51)	18% (7-44)	10% (2-32)	1.1 (0.6-3.9)

Figures represent median values (range)
\# Ratio of anti-leu 3: anti-leu 2
* P <0.01 Wilcoxon unpaired test

There was no linear correlation between the values obtained from the blood or tumours. A reversal of the anti-leu 3: anti-leu 2 ratio in the tumours, as reported by other authors using immunohistomchemistry has not been confirmed, but significant numbers of anti-leu 11 positive cells (NK cells) have been demonstrated within the tumours, a finding not previously reported. This technique represents a quick and easy way of analysing the sub-populations of lymphocytes within tumours, but has limitations of its own, which are discussed.

148. **HETEROGENEITY OF EXPRESSION OF MAJOR HISTOCOMPATABILITY ANTIGENS & TUMOUR ASSOCIATED ANTIGENS IN COLORECTAL TUMOURS**

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Expression of HLA/ABC and HLA/DR was measured by immuno-fluorescence and flow cytometry on colorectal carcinomas. There was vast heterogeneity in expression which, if related to tumour progression, could provide a biological classification. This would be distinct from clinicopathology as there was no correlation between expression and pathological staging or histological grade. Loss of HLA/ABC was associated with loss of tumour associated antigens, propensity for metastases and *in vitro* division. Expression of HLA/DR was associated with expression of tumour associated antigens and DNA aneuploidy. However cells in early *in vitro* culture were HLA/DR negative. Perhaps colorectal tumours are maintained and seeded by cells which lack histocompatibility antigen and are not recognised by the immune system.

149. ISOLATION AND CHARACTERIZATION OF A HUMAN MONOCLONAL ANTIBODY RECOGNISING MURINE IgG_2b IMMUNOGLOBULIN

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A colorectal cancer patient was injected with two radiolabelled monoclonal antibodies for detection of suspected recurrent disease. Blood was taken seven days after the final injection and the lymphocytes were fused with mouse myeloma, P3NSI. A stable hybridoma secreting a human IgM monoclonal antibody which recognised only one of the two injected antibodies was isolated. Detailed characterization revealed that it recognised an epitope unique to mouse IgG_2b immunoglobulin. Further studies will determine if other murine immunoglobulin subclasses produce a similar humoral response which could be a serious limitation in prolonged use of monoclonal antibodies for therapeutic applications.

150. EFFECT OF RECOMBINANT INTERFERON-GAMMA TREATMENT OF RAT TUMOUR CELL LINES ON NK, MACROPHAGE AND T CELL KILLING

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Treatment of rat tumour cell lines with recombinant interferon-gamma induces/augments class I MHC antigen expression, and reduces susceptibility to NK lysis. The effect of interferon-gamma treatment of target cells on susceptibility to macrophage and CTL killing has also been investigated, to elucidate further the basis of modification of target cell sensitivity by interferon.

Interferon-gamma treatment of tumour target cells has no effect on their susceptibility to macrophage killing. However, allogeneic CTL and T cells from syngeneic tumour-immune rats kill interferon-gamma treated tumour target cells more effectively. These results show that the modification of NK lysis by interferon is not due to a general effect on target cell sensitivity, but results from changes in target cell recognition.

151. STRONG CYTOLYTIC ACTIVITY AGAINST TUMOUR CELLS EXERTED BY $CD3^+$ $CD4^-$ $CD8^-$ $WT31^-$ CLONES CAN BE ENHANCED BY ANTI-CD3, ANTI-CD16 (IgG-FcR) MAb, IFNß & rIL2

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A minor population (2%) of normal $CD2^+$ $CD3^+$ human T lymphocytes lack both CD4 and CD8 antigens. $CD2^+$ $CD3^+$ $CD4^-$ $CD8^-$ clones derived from these lymphocytes like $CD2^+$ $CD3^-$ NK cell derived clones, exert strong cytolytic activity against a variety of so-called NK susceptible and nonsusceptible tumour target cells. In addition, some $CD3^+$ $CD4^-$ $CD8^-$ clones can also exert antibody dependent cellular cytotoxicity (ADCC), and express the CD16 antigen (IgG-Fc receptor). Both, ADCC activity and CD16 antigen expression are lower in $CD3^+$ $CD4^-$ $CD8^-$ than in $CD2^+$ $CD3^-$ NK cell derived clones. The lytic activity of $CD3^+$ $CD4^-$ $CD8^-$ clones can be blocked by anti-CD3 MAb and can be augmented by both anti-CD3 and anti-CD16 MAb. Lytic activity of $CD3^+$ $CD4^-$ $CD8^-$ and $CD2^+$ $CD3^-$ NK cell derived clones is considerably enhanced by 3 hr incubation with rIL2. Six out of 7 $CD3^+$ $CD4^-$ $CD8^-$ clones, like CD3 cloned NK cells exerted strong non-specific lytic activity against a variety of tumour cells. Thus, $CD3^+$ $CD4^-$ $CD8^-$ a $CD16^+$ cells share several features with $CD3^-$ NK cells. Our results also indicate that although $CD3^+$ $CD4^-$ $CD8^-$ clones react with 5 preparations of anti-CD3 MAb, these clones do not express a "classical" $CD3^+$/Ti antigen receptor complex because the framework epitope identified by the WT31 MAb is lacking. It will be discussed whether $CD3^+$ $CD4^-$ $CD8^-$ and $CD2^+$ $CD3^-$ clones are derived from the same or from different cell lineages.

152. SUPPRESSION OF TUMOUR ALLOGRAFT REACTIONS INDUCED BY SPECIFIC T-CELL FACTORS

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PC1-F, an antigen-binding T cell factor specific for picryl chloride (PC1) can transfer into native recipient mice the ability to mount, upon PC1 challenge, an immediate-hypersensitivity like response that is an obligatory initial step in the development of a DTH-response. It was recently shown by Ptak and Askenase, that injection of PC1-F also induces a feedback circuit, ultimately resulting in suppression of production of PC1-F, and thus suppressing DTH to PC1. This form of regulation appeared to be antigen-non-

specific as PCl-F suppressed not only DTH to PCl, but also to other antigens. This form of regulation did not effect antigen specific induction of lymphokine production, or of help for antibody production.

We now report that injection of PCl-F is also capable of inducing suppression of hypersensitivity responses to allogeneic and syngeneic tumour cells. In DTH responses to tumour cells, antigen (tumour) specific T cell factors play a role in initiation of the DTH response. Production of the tumour specific T cell factor, that renders macrophages cytotoxic to tumour cells (SMAF), can also be suppressed by injection of PCl-F. In addition rejection of allogeneic tumour cells was prevented or suppressed by injection of PCL-F. It is therefore tempting to conclude that the antigen-specific T cell factors PCl-F and SMAF belong to one and the same group of T cell products, that can be regulated by a form of feedback regulation that is directed to this type of T cell factors that are involved in the initiation of cellular immune responses.

153. **ROLE OF LYMPHOCYTE ACTIVATION IN THE GENERATION OF RELAPSING AND REMITTING EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS**

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We have found that a monoclonal antibody (anti-Tac) which binds to the human IL-2 receptor also reacts with guinea pig T cells. Using this reagent we have found that fluctuations in peripheral activated T cell levels occur with disease status in relapsing and remitting EAE and that the peripheral level of activated T cells correlates with, and perhaps controls, changes in activated T cell levels in the cerebrospinal fluid. The meningeal compartment contains fewer total T cells whose levels also fluctuate with disease state but even in adjuvant control animals elevated levels of activated T cells are present in this compartment.

154. **RE-EVALUATION OF THE PATHOGENESIS OF EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS (EAE) IN THE RAT**

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The mechanisms by which $CD4^+$ T cells induce damage in the central nervous system (CNS) in EAE are unknown and it is not clear whether the other cell types, found infiltrating EAE lesions, play an essential role in pathogenesis. Dogma would have it however, that together, these cells may be participants in a DTH reaction within the CNS. Data to be

presented strongly challenge this view as we now provide in vivo evidence which suggest that the encephalitogenic effector cells are IL-2 receptor positive, $CD4^+$ T lymphocytes which function independently of other infiltrating leukocytes, and that it is damage to the vasculature (either directly or indirectly) by these cells, that is ultimately responsible for the clinical signs of EAE.

155. **ISOLATION OF T CELLS SPECIFICALLY REACTIVE TO ACETYLCHOLINE RECEPTOR FROM PATIENTS WITH MYASTHENIA GRAVIS**

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Peripheral blood lymphocytes from 62 myasthenia gravis patients were assayed for ^{3}H-thymidine uptake in the presence of purified Torpedo (electric fish) acetylcholine receptor (T-AChR). Stimulation indices (SI) of 3-10 (mean 5.5) were obtained in four. Attempts to raise T cell lines using IL2 and T-AChR were successful only in these four patients, from whom a total of nine lines were obtained. Most phenotypes were >80% $T3^+$, $T4^+$, but two lines from different patients were more heterogeneous (20% $T4^+$, >50% $T8^+$). Line cells reacted similarly to T-AChR and to its alpha-chain.

Two T-AChR specific clones (phenotype 83% $T4^+$, 0% $T8^+$, 78% Ia^+) were isolated from one line, and SI for T-AChR and alpha-chain were 40-50. The fine specificity and HLA restriction of these clones are being studied.

156. **T-CELL CLONES PREPARED FROM RHEUMATOID ARTHRITIS SYNOVIAL MEMBRANE**

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T cell clones, from the synovial membrane of a R.A., were prepared and expanded using monoclonal antibody to the CD3 complex. 60 clones were obtained. The phenotype of 12 randomly chosen clones are positive for CD3, CD4, 4B4 and negative for CD8, 2H4, Leu 7 and Leu 11. As products of these cells can have a relevant role in the pathogenesis of R.A. we studied the presence of these lymphokines: gamma interferon, LT and TNF in the supernatant of these cells. High levels of gamma interferon were found range 11-2442 U/ml mean 348 + 393. Lower, but detectable, amounts of TNF

and LT were found in some of the supernatants. This is of particular interest as these lymphokines can synergize in producing tissue damage and in the induction of class II molecules.

157. **INTRATHYROIDAL T CELL CLONES FROM PATIENTS WITH AUTOIMMUNE THYROID DISEASE**

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T cell clones were isolated from within the thyroid of patients with autoimmune hyperthyroidism and thyroiditis. These were analyzed for phenotype, reactivity and function and were found to be significantly different by all criteria. Graves' disease gave predominatnly CD4/4B4 clones, whereas thyroiditis clones were 65% CD8 with the CD4 population divided equally between 4B4 and 2H4. Both disease types gave rise to thyroid specific clones, but to different Ags. Functionally, Graves' disease T cell clones were 65% helpers, with no cytotoxic clones found, whereas the majority of thyroiditis clones were cytotoxic. It is likely that the disparate nature of the T cells from these diseases reflects the situation in vivo, thereby determining the pathogenesis.

158. **ACTIVATION OF AUTOREACTIVE ANTI-THYROGLOBULIN T CELL HYBRIDOMAS: REQUIREMENT FOR IODINATED THYROGLOBULIN**

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We have recently described the production and characterization of mouse thyroglobulin (Tg)-specific autoreactive T cell lines and hybridomas, some of which cross-react with human Tg. Using a panel of human Tgs, we found that two independently-derived T cell hybridomas, ADA2 and CH9, were only activated by Tgs with a molar ratio of at least 0.3 thyroxine (T4) residues per Tg molecule. As a second approach to investigating the role of iodine in autoantigen recognition, we prepared non-iodinated Tg from mice treated with aminotriazole (ATA), which blocks the peroxidase-catalysed organification of iodide. Tg from ATA-treated mice was unable to stimulate ADA2 or CH9, whereas Tg from untreated littermates stimulated both hybridomas, indicating a dependence on iodination for activation of these T cell hybridomas. (A third T cell hybridoma, C4, was activated by Tg from both normal and ATA-treated mice). However, since ADA2 and C4 were not stimulated by T4 coupled to albumin, or to fully iodinated Tgs from certain species, this indicates that the requirements for activation include other residues as well.

The molecular basis for this specificity is not yet clear, but may involve considerations of antigen processing or antigen-Ia interactions. An alternate hypothesis, which is now under investigation, is that iodinated residues may constitute dominant epitopes for the activation of anti-Tg T cells.

159. **EXPRESSION OF T LYMPHOCYTE ANTIGENS WITHIN MULTIPLE SCLEROSIS LESIONS**

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Post-mortem brain tissue from six patients with multiple sclerosis, including four who were relatively young and had a recent history of aggressive disease, was examined for the presence of immunocompetent cells using monoclonal antibodies and immunoperoxidase staining. Forty-one lesions were found to feature macrophages containing lipid and/or protein degradation products of myelin, indicating the occurence of recent or ongoing demyelination. T lymphocytes were identified (using Leu 4) in thirty-six lesions but found in significant numbers in only thirteen. Signficantly fewer cells were labelled with two other pan-T cell antibodies recognising different cell surface structures, Leu 1 and OKT 11 ($p<0.02$). Examination of the expression of helper and suppressor T cell antigens revealed approximately equal numbers of positive cells in the plaque and periplaque regions, although the sum of these sometimes greatly exceeded the number of cells labelled by pan-T cell antibodies.

NUMBERS OF CELLS RECOGNISED BY ANTI T-CELL ANTIBODIES IN MS PLAQUE AND PERIPLAQUE REGIONS (MEAN ± S.D.)

	Total T cells		Helper T cells	Suppressor T cells	
Leu 4	Leu 1	OKT 11	Leu 3a	Leu 2a	OKT 8
335	118	183	202	232	172
±157	±63	±110	±115	±160	±119

Reduced expression of T cell antigens has previously been reported to occur also in peripheral blood lymphocytes in MS. This suggests that identification of T cells with monoclonal antibodies may fail to detect all of the T cells present. The consequences of altered expression of these functionally important cell surface molecules remain unknown at present.

160. **MOLECULAR CLONING AND CHARACTERISATION OF BOVINE CLASS I MHC GENES**

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An investigation of the genes encoding bovine lymphocyte antigens (BoLA) has been undertaken. Lambda GT10 cDNA library was constructed from bovine liver mRNA. Of 250,000 clones, one proved positive by hybridisation to a HLA 27 probe, with an insert size of 1.2kb. This clone was judged to be an authentic bovine class I cDNA clone by hybridisation to total RNA from lymphocytes and liver, generating an identical hybridisation profile to the HLA27 probe.

Using the 1.2kb cDNA clone as a probe the lambda GT10 library was re-screened and additional positives were obtained. Characterisation and sequencing of these clones is under way at present.

161. **INTERACTION BETWEEN CYTOMEGALOVIRUS & CLASS I HLA MOLECULES; ROLE IN VIRAL INFECTIVITY**

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We have previously demonstrated that cytomegalovirus (CMV) binds the host protein β_2 microglobulin (β_2m) from body fluids or from cell culture media. We have now shown that the addition of human β_2m, or a fraction of foetal calf serum corresponding to bovine β_2m, enhances the infectivity of CMV grown in cell culture. Furthermore CMV and β_2m compete for the same binding sites on fibroblasts. In addition CMV was found to bind to a significantly greater degree to Raji cells expressing Class I HLA antigens, than to Daudi cells lacking such expression.

Our results demonstrate that CMV can use Class I HLA molecules as a virus receptor. We proposed that when coated with β_2m, CMV has the capacity to displace β_2m from the Class I HLA heavy chain-β_2m dimer on the cell surface and bind to cells. The fact that β_2m enhances infectivity suggests that such binding leads to productive infection of cells.

162. **PURGING WITH MONOCLONAL ANTIBODIES McAbs AND COMPLEMENT C IN AUTOLOGOUS BONE MARROW TRANSPLANTATION ABMT: SELECTION OF THE OPTIMAL REAGENTS**

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ABMT is used as a rescue following intensive radio-chemotherapy in patients with acute leukaemia and lymphoma when a HLA compatible donor is not available. The risk of infusion of residual malignant cells can be reduced by collecting the bone marrow in complete remission and by the removal of residual blasts by purging procedures. C fixing McAb-s are simple and economical reagents which can be used for the latter purpose. The efficacy of the cytoreduction achieved depends upon the selection of the optimal combination of McAb-s and C for each individual patient by using sensitive techniques capable of detecting very small numbers of residual neoplastic cells. First, we developed methods to identify very low numbers of residual malignant cells by using independent markers such as nuclear TdT or B cell antigens. Second, we have identified McAb-s with both rabbit and human C fixing capacity and the highest degree of reactivity with individual cases of leukaemia and lymphoma. We tested more than 100 samples of patients with common acute lymphoblastic leukaemia (cALL), T-ALL, B-ALL and B cell non Hodgkin lymphoma (B-NHL) at presentation or in relapse. The efficacy of cytolysis was calculated to be as low as <0.001% of residual malignant cells. The results from this study indicate that >99.99% of lysis can be achieved in approximately 75% of cases by using the following combinations of murine McAb-s and rabbit C: RFAL3 (IgM class, CD10) + SB4 (IgM class, CD19) for common and null ALL; RFT2 (IgG2 class, CD7) for T-ALL; RFB7 (IgM class, pan B) + SB4 for B-NHL. RFAL3, SB4 and RFB7 can also fix human C. The antigens identified by these Ab-s appear to be expressed not only on resting populations but also on the surface of leukaemic cells which are proliferating as indicated by double staining studies in combination with a McAb anti-BrdU. Additional investigations employing leukaemic "clonogenic" assays are also performed. The clinical experience with these reagents will be discussed.

163. **DEOXYGUANOSINE INDUCED THYMIC ALLOGRAFT SURVIVAL IS ASSOCIATED WITH DEPLETION OF DONOR-TYPE Ia POSITIVE THYMIC MEDULLARY DENDRITIC CELLS**

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Thirteen day foetal mouse thymus treated with deoxyguanosine (dGuo) undergo lymphoid depletion and survive on allogeneic transplantation despite continued epithelial donor-type Ia

expression. Investigation of the effect of dGuo on antigen presenting, Ia positive thymic dendritic cells (T-D.C) has now revealed that donor Ia expressing T-DC are identifiable by rosette formation in cell suspensions from normally cultured CBA ($H\text{-}2^k$) foetal thymic grafts three weeks after transplantation into Balb/c nudes ($H2^d$). T-D.C are, however, severely depleted or undetectable in comparable dGuo pretreated grafts. T-D.C depletion therefore correlates with dGuo induced allograft survival and emphasises that the significance of Ia antigen in allograft rejection depends upon the type of cell upon which it is expressed.

164. **ALLOREACTIVE CYTOTOXIC CELLS GENERATED IN VITRO DETECT BOLA W6 SUBGROUPS**

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Bovine MHC class I antigens are known as BoLA antigens. They have been defined in cattle using specific antisera generated in cattle immunised with lymphocytes or skin grafts from non MHC identical animals. We have shown that alloreactive cytotoxic T cells (CTL) are generated in mixed lymphocyte culture against cells bearing subgroups of BoLA w6, as well as BoLA w4, w10 and w16. The killing of the four subgrops of w6': w6.1, w6.2, 6.3 and 6.4 by CTL was very specific. Primary cultures were restimulated at weekly intervals with irradiated stimulator cells and tested in a ^{51}Cr release assay on T.annulata infected cell lines as targets. Generation of CTL was accelerated in animals that had been previously primed in-vivo by skin grafting.

Where CTL were generated against two BoLA alleles at the same time one of which was a w6 subgroup there was similar CTL activity against both alleles when 6.4 and w16 or w6.1 and w4 were compared. In two generations against w10 and either w6.1 or w6.2 there was significantly more killing of w10 targets than those with the w6 subgroup.

165. **EXPRESSION OF CLASS II MAJOR HISTOCOMPATIBILITY ANTIGENS BY KERATINOCYTES DURING CONTACT SENSITIVITY**

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Normal murine skin keratinocytes do not express MHC class II (Ia) antigens. We have observed, however, that the epicutaneous application of sensitising chemicals at both the induction and elicitation phases of contact allergy results in the appearance of strongly Ia-positive (both I-A

and I-E) keratinocytes within 5 days of exposure. It is of interest that, under the conditions employed, irritant, non-sensitising chemicals which result in similar levels of local erythema and induration fail to cause the induction of class II antigen expression. The relevance of these observations to the induction and regulation of contact sensitisation is currently being investigated.

166. **DENDRITIC CELLS OF STIMULATOR HAPLOTYPE PRESENT ALLOANTIGENS TO T LYMPHOCYTES DEPLETED OF SYNGENEIC ANTIGEN PRESENTING CELLS**

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Recent work has suggested that alloantigens are presented by antigen presenting cells (APC) of the responder haplotype in the induction of a mixed leucocyte response (MLR)[1] and skin graft rejection[2]. We used CBA mouse spleen cells to stimulate proliferation in B.10 lymph node nylon wool purified T cells. Low numbers of CBA dendritic cells (DC) stimulated in MLR, the response being unaltered by the depletion on metrizamide of B.10 DC from the responding population. CBA macrophages depleted of DC were poor stimulators. Responding B.10 T cells showed allospecificity when restimulated by CBA compared with B.10 and Balb/c DC. These results suggest that alloantigens are presented by stimulator DC without a requirement for responder APC. Experiments using additional methods to deplete of DC are in progress.

1. Baxevanis CN et al. Eur. J. Immunol. 1986, 16, 361
2. Sherwood RA et al. Eur. J. Immunol. 1986, 16 569

167. **EFFECT OF LONG TERM PHENOBARBITONE ADMINISTRATION ON CYCLOSPORIN A-INDUCED NEPHROTOXICITY & HEPATOXICITY IN RENAL ALLOGRAFTED RATS**

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Nephrotoxicity, and to a lesser extent hepatotoxicity are clinically worrying side-effects in organ transplant patients receiving cyclosporin A (CsA). We have previously demonstrated that phenobarbitone (40 mg/kg), an inducer of the hepatic P-450 mono-oxygenase enzyme system alleviates CsA-induced nephrotoxicity in both normal rats and in those experiencing graft-versus-host disease.

The effect of phenobarbitone (40 mg/kg) on CsA-induced nephrotoxicity was investigated in renal allografted F_1 hybrid (DA x LEW) LEW rats receiving 40 mg/kg CsA. Non-immunosuppressed rats rejected their transplanted kidneys by day 9 whereas these receiving CsA alone died between days 7-168 (mean=37, n=16) and those receiving phenobarbitone and CsA in combination died between days 7-95 (mean=30, n=14). Light microscopy revealed a similar extent of renal proximal straight and convoluted tubular damage in both the CsA treated groups. In addition, liver necrosis was evident in CsA treated rats receiving phenobarbitone suggesting that long-term phenobarbitone administration may enhance CsA induced hepatotoxicity. The results indicate that the combination of surgical stress, CsA and long-term phenobarbitone treatment predispose these animals to enhanced hepatotoxicity.

168. **CELL SURFACE MOLECULES INVOLVED IN NK RECOGNITION BY CLONED CYTOTOXIC T LYMPHOCYTES (CTL)**

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Cloned CTL cultured for up to 2 months in lectin-free medium acquired high levels of NK activity when treated with IFN and promiscuous lytic activity when treated with IL-2. Cold target competition analysis showed that the development of NK activity was associated with acquisition of novel binding activity for NK-sensitive targets, whereas promiscuous lytic activity was associated with acquisition of binding activity for both NK-sensitive and NK-resistant targets. Ag-specific cytolysis was inhibited by antibodies to Ly-2, Ly-5, LFA-1, and TCR. By contrast, NK and promiscuous lytic activity in the same cells was resistant to inhibition by anti-Ly-2 and anti-TCR. NK activity was expressed normally against a variant NK-sensitive line lacking all MHC antigens.

These results show that NK and promiscuous lytic activity in CTL occur without involvement of residual mitogen, Ly-2, or TCR, and are most likely mediated through novel and distinct receptor systems.

169. **SUPPRESSIVE EFFECTS ON IMMUNOGLOBULIN SYNTHESIS OF LEU 11 & LEU 7 POSITIVE NK CELLS**

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Leu $7a^+$ natural killer (NK) cells have been reported to exert immunoregulatory effects *in vitro*. However, it has been shown that most NK cell cytotoxic activity resides within the Leu 11 (CD 16)$^+$ population. We thus compared

the effects of Leu $11b^+$ and Leu 7^+ peripheral blood mononuclear cells (PMBC) on pokeweed mitogen induced immunoglobulin production. When PBMC from normal donors were depleted of Leu 7^+ or Leu $11b^+$ cells by complement lysis or by fluorescence activated cell sorting (FACS), immunoglobulin synthesis was markedly enhanced as compared with appropriate controls and the enhancement was significantly greater following Leu 11^+ depletion. Addition of Leu $11b^+$ cells (obtained by FACS) to PBMC, resulted in marked inhibition of immunoglobulin synthesis whereas addition of similar numbers of Leu 7^+ cells was less inhibitory. Furthermore, addition of anti-Leu 7 or anti-Leu 11b antibody alone to PBMC reduced immunoglobulin synthesis; again this effect was more marked with anti-Leu 11b. These results show that Leu $11b^+$ NK cells mediate suppression of Ig synthesis, and suggest that suppression can be induced by anti-Leu 7 and Leu 11b binding alone.

170. **GUT EPITHELIAL CELL Ia ANTIGENS STIMULATE ALLOGENEIC "MIXED LYMPHOCYTE"-TYPE RESPONSES**

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Ia antigens are distributed throughout the small intestine on fully-differentiated absorptive enterocytes and have been shown to restrict the *in vitro* presentation of soluble protein antigens by enterocytes to T cells. The capacity for Ia-bearing enterocytes to stimulate syngeneic and allogeneic "MLR" like responses was investigated in co-culture of T-cells and enterocytes with WF($RT1^u$), F344($RT1^1$), BUF($RT1^b$) and ACI($RT1^a$) rats. No T cell proliferation was detected in syngeneic co-cultures, even with the addition of exogenous IL-2 to expand minimally reactive T cell clones. However, significant responsiveness was detected after allogeneic co-culture. In general, F344 and BUF enterocytes acted as good stimulators, whereas F344 T cells responded poorly to all allogeneic enterocytes.

171. **ANTI-MHC CLASS II ANTIBODIES AND OKT3 ACTIVATION OF PBL: DISSECTION OF THE INHIBITION MECHANISM**

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Several anti-MHC class II (DP, DQ and DR) monoclonal antibodies have been tested to study their effect on PBL activation by OKT3. A differentiated effect, ranging from none to complete inhibition of activation, was found,

suggesting the involvement of different epitopes of MHC molecules in the process leading to PBL proliferation. Blocking of activation appears to be specific and not Fc-mediated.

Kinetics experiments showed that the early steps of proliferation are affected by the inhibiting antibodies. A reduced expression of Tac receptor was also observed. This is not related to a possible interaction of the Tac receptor with the class II antibodies, as demonstrated by competition experiments with an anti-Tac antibody.

In order to define the cell target of the inhibition, purified populations were prepared from PBL and tested. The results indicate that both monocytes and T cells are possibly involved.

A likely role of lymphokines in the mechanism of inhibition is also suggested by experiments showing a reduced production of these molecules. All these observations will be dealt with in an attempt to design a pattern of the inhibition exerted by MHC class II antigens on OKT3 lymphocyte activation.

172. **NEW MONOCLONAL ANTIBODY (BU-18) WHICH IDENTIFIES AN ANTIGEN RESTRICTED TO PLASMA CELLS & SECRETORY EPITHELIA**

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We previously described to this Society a group of Moabs reactive with plasma cells, other lymphoid cells and secretory epithelia . The antibody we now report detects intracellular antigen in plasma cells and secretory epithelia but is unreactive with other haematopoietic cells.

The reactivity pattern was established by FACS analysis on a wide range of cell lines and immunofluorescent staining of fixed cell preparations and cryostat sections of lymphoid tissues, secretory and non-secretory epithelia.

The new antibody should be useful for immunocytochemical identification of plasma cells and in the study of antigens associated with secretory apparatus.

173. **CHARACTERISATION OF 2 HUMAN X MOUSE HYBRID CELL-LINES EXPRESSING HUMAN CD4**

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The CD4 molecule is a 55-62 KD cell-membrane polypeptide expressed by human helper T cells, recognised by monoclonal antibodies such as Leu 3 and OKT4. It appears to function as a receptor for the invariant portion of HLA class II. To study CD4 expression we have isolated 2 human x mouse somatic cell hybrids, prepared by fusing an EBV-transformed human cell-line and a ALL with the mouse T lymphoma, BW5147. The two hybrids (TF42 and TF53.1) were immunoselected with OKT4 on the cell-sorter to retrieve CD4 + variants. Complete isoenzyme and chromosome analyses showed that TF53.1 contained 2 and the TF42 13 human chromosomes. Expression of CD4 (OKT4) showed concordance with chromosome 12 but not 11, or X chromosome as revealed by analyses with various single copy, chromosome-specific DNA probes. After sorting with OKT4 both hybrids were stable for CD4 expression in culture. CD4 positive variants of TF42 contained multiple copies of chromosome 12 suggesting gene amplification. CD4 + and CD4 - clones obtained from TF42 and uncloned TF53.1 were used to screen a panel of CD4 MoAB, all of which showed similar patterns of reactivity by flow cytometric analysis. These hybrids should be useful in defining various epitopes of the CD4 molecule, studying the function of CD4 and determining what influences CD4 expression.

BASEMENT FOYER

MHC & TRANSPLANTATION

Po.1 APPEARANCE OF CELLS BEARING THE INTERLEUKIN-2 RECEPTOR AND CHANGES IN T-CELL SUBPOPULATIONS IN THE BLOOD OF PATIENTS AFTER CARDIAC TRANSPLANTATION; CORRELATION OF THESE EVENTS WITH REJECTION

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Rejection of recipients of cardiac allografts is diagnosed by the presence of an infiltrate in cardiac biopsies. Whether events in the graft are preceded, or accompanied by changes in the blood are not known. Here we have used smears of whole blood and an APAAP technique to detect interleukin-2 receptor (IL-2) positive cells and ratios of T-helper/inducer to T-cytotoxic/suppressor cells in a longitudinal study of 10 patients after cardiac transplantation. All patients received azathioprine and Cyclosporine immunosuppression. Rejection was defined by histological assessment of the cardiac biopsy. In total, 16/30 (53%) rejection episodes were preceded within 3 days by an increase in the percentage of IL-2 receptor positive cells in the blood. Increases in the T4/T8 ratio preceded 15/30 (50%) rejection episodes. Using either parameter, 80% of rejection episodes were detected within 3 days of biopsy. These results are discussed in terms of the immunological mechanisms causing graft rejection and the possible use of these parameters to aid non-invasive diagnosis of rejection.

Po.2 ANTIBODIES TO LIVER MEMBRANE PROTEIN COMPLEX (LSP) IN PATIENTS AFTER BONE MARROW TRANSPLANTATION (BMT)

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Follow up sera from 19 patients with BMT were screened for anti-human LSP antibodies by ELISA. 3 patients did not present significant GVH reactions and were anti-LSP negative. Among the 13 cases with GVHD, 3 presented with anti-LSP occurring 12 to 19 months after the BMT: liver involvement was demonstrated by histology in all 3 cases. Acute viral hepatitis (AVH) occurred in 3 cases all anti-LSP positive 1 to 6 months after BMT. In conclusion, among 7 cases with biopsy proven hepatic GVHD, 4 had anti-LSP (1 presented also AVH) while among 4 patients with GVH without liver involvement, only 1 (with AVH) was anti-LSP positive.

Po.3 TRANSFUSION EFFECT ON GVHD AND LEUKEMIC RELAPSE IN HLA-MATCHED BONE MARROW TRANSPLANTATION

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The effect of red cell transfusions given shortly before bone marrow transplantation (MBT) was evaluated in 790 leukemic patients transplanted with marrow from an HLA-identical sibling donor. The group of 253 patients who received a transfusion shortly before BMT as judged by mixed cell types in red cell antigen and red cell enzyme assays at that time, had a similar incidence and severity of acute graft-versus-host disease (GVHD), but a significantly lower incidence of chronic GVHD (approximately 35%) compared to a group of 537 patients without such transfusions (48%, p=0.002). However, the first group had earlier and more relapses than the second group, making the survival between the groups not significantly different. In multivariate analyses, taking into account the risk factors for GVHD and relapse like: age, diagnosis, status at BMT and sex, these differences remained significant.

Po.4 ROLES OF HOST T LYMPHOCYTES AND NATURAL KILLER CELLS IN THE INTESTINAL PHASE OF MURINE GRAFT-VERSUS-HOST REACTION

[WITHDRAWN]

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SUBSTITUTION

OBJECTIVITY AND THE DYE EXCLUSION CYTOTOXIC CROSS MATCH ASSAY

D Talbot, A Stratton, A Givan, BK Shenton, G Proud & RMR Taylor

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Po.5 INDUCTION OF PROLIFERATIVE & DESTRUCTIVE GRAFT-VERSUS-HOST REACTIONS

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Several chronic disorders associated with small intestinal injury exhibit a similar pattern of villus atrophy, crypt hyperplasia and lymphocyte infiltration of the epithelium and are associated with local cell mediated immunity (CMI).

We have used the intestinal phase of the graft-versus-host reaction (GvHR) in (CBAxBALB/C)F_1 mice as a model of enteropathy due to CMI. 7 day old neonatal mice develop a proliferative GvHR, with NK cell activation and crypt hyperplasia but with no evidence of villus damage or specific cytotoxic T-cell (CTL) activity. The induction of GvHR in 2 day old mice results in a lethal disease. Before the onset of runting, around day 8, a transient peak in NK activity coincided with crypt hyperplasia while the appearance of CTL and villus atrophy corresponded to the development of runting.

These results are consistent with the idea that the proliferative enteropathy in GvHR is associated with a DTH response, whereas the destructive enteropathy characterised by villus atrophy is associated with CTL.

Po.6 CLASS II ALPHA CHAIN GENE RESTRICTION FRAGMENT LENGTH POLYMORPHISM (RFLP) IN ASIANS WITH INSULIN DEPENDENT DIABETES MELLITUS (IDDM)

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We have previously described Class II alpha chain gene polymorphisms which segregate with Caucasoid IDDM subjects, and suggested one of the genes involved in this disease is located within the HLA DQ region. In this study we have analysed 111 Indians living in Madras (58 with IDDM and 43 controls) to investigate whether the same RFLPs associate with diabetes. DNA was extracted from blood, and studied by Southern blot hybridisation techniques. Unlike Caucasoids no association of IDDM was seen with the DX alpha gene. Taq I digestion and hybridisation with a DQ alpha probe identified five bands. The allelic frequency of the 4.6 kb band was increased in IDDM (0.32 compared to 0.11 in controls; $p<0.001$). Furthermore 19% of patients were homozygous for DQ alpha 4.6 in contrast to none of the

controls (relative risk = 21). The DQ alpha DR3 related polymorphism was found to be in coupling with a DR alpha 4.5 kb fragment (detected by Bgl II digestion) in contrast to Caucasoids when it is associated with a 4.2 kb fragment. No differences in frequency of any other DQ alpha RFLPs were seen between diabetics and controls including that of a DR4 related band. In conclusion although different preferential allelic associations are seen in Indian compared to Caucasoid IDDM subjects, population studies confirm the DQ region to be relevant for the susceptibility of this disease.

IN VIVO IMMUNOLOGY

Po.7 EFFECT OF HISTAMINE & PROSTAGLANDIN E_2 (PGE_2) ON THE MICROCIRCULATION IN THE SKIN

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Histamine and PGE_2 are recognised as potent mediators in immunological inflammatory reactions. Their effect on cutaneous blood flow was measured with a laser-Doppler flowmeter in 6 normal human subjects. In the centre of the histamine reaction, blood flow rate increased rapidly and then fell exponentially: there was little inter-subject variation, the peak level was dose-independent but more prolonged at higher concentration. PGE_2 caused a longer-sustained rise in blood flow but with considerable intersubject variation: the duration of the peak flow rate was dose-dependent and the raised flow rate decayed more slowly than the histamine response. These microcirculatory responses are similar to those noted previously in the Mantoux reaction.

Po.8 COMPARISON OF ^{51}CHROMIUM AND HOESCHT 33342 FOR MONITORING LYMPHOCYTE MIGRATION IN VIVO

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In the LEW (RTI^1) to DA (RTI^a) rat we have previously demonstrated long term renal allograft survival with normal spleen cells and abrogation of this effect by heat treatment or irradiation. Migration of these three lymphocyte populations in recipient animals was investigated quantitatively by ^{51}chromium labelling, and qualitatively by H33342, a fluorochrome dye, which allow visualisation of allogeneic LEW cells on cryostat sections of host (DA)

spleen. Both techniques demonstrate that heat treated cells do not localise in the spleen. ^{51}Cr labelling shows no difference between irradiated cells and normal cells, whereas H33342 clearly demonstrates destruction of the irradiated cells within the host spleen within 24 hours.

Po.9 CHARACTERISATION OF SHEEP INTERLEUKINS & THE FATE OF INTERLEUKINS IN-VIVO

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We are using cannulated peripheral lymphatics in the sheep to characterise the kinetics of IL-1 and IL-2 release during antigen challenge of a peripheral lymph node. Whole afferent and efferent lymph has been collected following an intradermal challenge with soluble protein antigen and separated into cells and lymph fluid by centrifugation. Total RNA extracted from the cells is currently being analysed for interleukin mRNA content by Northern blotting using mouse IL-1 and human IL-2 cDNA. The lymph fluid is being fractionated in an attempt to isolate sheep IL-1 and IL-2. Afferent and efferent lymph collected 24 and 48 hours after antigen stimulation contain material that causes proliferation of Con A-induced blast cells. This material is being further fractionated by gel filtration and HPLC.

We are also examining the fate of human recombinant IL-1 and IL-2 following administration via an afferent lymphatic to a resting or antigen-stimulated peripheral lymph node. The chemical and biological half-life of administered material is being assessed.

Po.10 THE MIGRATION PATTERN OF VIRGIN B CELLS ON LEAVING THE BONE MARROW DIFFERS FROM THAT OF RECIRCULATING B CELLS

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Cell transfer studies have indicated that both B cells recently produced in the bone marrow, and mature recirculating B cells, can be activated in the early phases of thymus dependent antibody responses. However, sustained antibody production appears to result from repeated activation of memory B cell clones without further B cell recruitment[1]. To investigate this further, we have compared the migration of recirculating B cells (RB) with that of B cells from the marrow of rats depleted of recirculating cells (VB). Transfers have been made between

rats of congenic strains which differ in their kappa allotype. The capacity of transferred cells to enter the spleen and lymph nodes of recipients was assessed by immunohistology. At 2 hours after intravenous transfer, both RB and VB were mainly found in the periarteriolar lymphocytic sheath and the red pulp of the spleen, and in the perilymphatic and corticomedullary areas of the paracortex of lymph nodes. Increased numbers of VB were found in these sites at 8 hours, but relatively few VB were seen in follicles in either lymph nodes or spleen by 24 hours post transfer. In contrast RB were mainly located in follicles at 8 hours, 24 hours and one week after transfer. These findings suggest that both VB and RB gain access to antigen on interdigitating cells in extrafollicular areas of secondary lymphoid organs. However, only RB appear to encounter antigen on follicular dendritic cells.

1. Gray D, MacLennan ICM & Lane PJL (1986). Virgin B cell recruitment and the lifespan of memory clones during antibody responses to DNP-Hemocyanin. Eur. J. Immunol. (in press).

Po.11 $L3T4^+$ MURINE T-CELLS ARISE FROM $LYT2^+$ PRECURSORS DURING THYMIC ONTOGENY IN VIVO

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The adult murine thymus contains four subpopulations of thymocytes defined by the T-cell surface antigens L3T4 (the marker of helper T-cells) and Lyt2 (the marker of cytotoxic/suppressor T-cells): $L3T4^+$ $lyt2^-$ and $L3T4^-$ $Lyt2^+$ "single positives", $L3T4^+$ $Lyt2^+$ "double positives", and $L3T4^-$ $Lyt2^-$ "double negatives". In order to understand the sequence of intrathymic events that make up T-cell ontogeny, it is vital to determine the lineage relationships among these subpopulations. In particular, the status of double positives has long been controversial. Some hypotheses hold that this subpopulation contains precursors of the more mature single positives. Others propose that double positives are "dead end cells" that all die in situ, perhaps because they have been rejected by some selective process. A great deal of suggestive evidence has been cited in support of these various hypotheses, but direct evidence has been lacking. Presented here is the first direct evidence that the $L3T4^+$ $Lyt2^-$ single positive subpopulation is derived entirely from double positives *in vivo*. Radiation bone marrow chimaeras were made by injecting a mixture of CBA (Lyt2.1, Thy1.2) and AThy1.1

(Lyt2.2, Thy1.1) bone marrow cells into irradiated hosts, and subsequently injected with allele-specific anti-Lyt2 monoclonal antibodies chronically over a four-week period. The results show that anti-Lyt2.1 injection selectively eliminates the production of $Ly2^+$ and $L3T4^+$ single positive subsets derived from CBA bone marrow cells, while anti-Lyt2.2 injection eliminates both single positive subsets derived from AThy1.1 bone marrow cells. This finding demonstrates that $L3T4^+$ $Lyt2^-$ T cells develop from an $Lyt2^+$ precursor, presumably a double positive thymocyte.

CELL DIFFERENTIATION & ACTIVATION

Po.12 ONE-PLATE MICROFLUORIMETRIC PEROXIDE RELEASE ASSAY FOR CELLULAR ACTIVATION

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The ability of lymphokine-induced mononucleocytes to release hydrogen peroxide on secondary stimulation has been correlated with antibacterial potency. This property has been used to assay T-cell supernatants for Macrophage Activating Factor (MAF) activity.

We have devised an integrated assay which allows activation of adherent or non-adherent cells with test material, measurement of peroxide release and cellular quantitation in the same microtitre plate.

Peroxide release is estimated by scopoletin oxidation followed, after suitable link steps, by the DNA-binding dye H33258. The assay requires a single machine at preset wavelength, and has been used successfully with T-cell clone, peripheral blood and human spleen cell supernatants.

Po.13 MURINE CYTOMEGALOVIRUS-INDUCED CHANGES IN SPLEEN CELL PRODUCTION OF, & RESPONSE TO, INTERLEUKIN 2

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Mice undergoing murine cytomegalovirus infection show a depressed response to the T cell mitogen Concanavalin A (Con A).

Investigation of the mechanism has shown that 6 to 10 days post infection (after peak virus replication in the spleen, but coinciding with splenomegaly) spleen cells make little

interleukin 2 (IL 2) in response to Con A, and do not recover the ability to respond with time in culture. Low IL 2 activity is mainly associated with the non-adherent population, but the proportions of T cell subsets, as determined by monoclonal antibodies to Lyt 1, Lyt 2 and L3/T4, are largely unchanged. There is no evidence of suppression of IL2 production by either cell-interaction or by soluble inhibitor.

Conversely spleen cells at the same time post infection are able to respond to preformed IL 2, suggesting that at least a proportion bear IL 2 receptors.

Po.14 PRIMARY & SECONDARY IN VITRO RESPONSES OF MURINE T CELLS TO AUTOLOGOUS AND CROSS-REACTIVE ERYTHROCYTES

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Mice can be induced to make anti-erythrocyte autoantibodies by immunisation with rat red blood cells (RBC). Since autoreactive helper cells were assumed to be lacking, it was proposed that the help required for autoantibody production was provided by rat specific T helper cells. However, we show here that mouse RBC reactive T helper cells are present in the normal mouse and can be activated by immunisation with rat or mouse RBC. Both mouse/rat cross-reacting and mouse speicific T helper cells can be detected. These findings show that deletion is not complete and that immunoregulatory control must be involved in the prevention of anti-RBC autoimmunity.

Po.15 ANALYSIS OF HUMAN LYMPHOCYTE TRANSFORMATION RESPONSES TO GRADED DOSES OF MITOGENS BY CURVE FITTING

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Complete lymphocyte transformation dose-response curves to graded doses of phytohaemagglutinin (PHA) or concanavalin A (Con A) are bell shaped when plotted using a log-dose scale. Subsequent between-patient-group analysis of such dose-response curves can be complex, so we have derived a mathematical model of this phenomenon. This provides estimates of the magnitude of the peak response, the dose of mitogen necessary to elicit the peak and an estimate of the range of mitogen responses that produce a response. The

model was more accurate in describing responses elicited by Con A than those by PHA. The model may be of use in comparing sets of control and patients' transformation responses, as a criterion for rejecting suspect data, or for the quantitation of immunoregulatory factors.

Po.16 LYMPHOCYTE TRANSFORMATION TO CON A & PHA: ARTEFACTUAL RESULTS FOLLOWING LYMPHOCYTE SEPARATION ON FICOLL-PAQUE COLUMNS

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Lymphocytes for use in transformation tests are first separated from peripheral blood by gravity sedimentation or, more commonly, by density sedimentation using the method of Boyum. Variations in results according to the method of isolation used are well documented, but have not been further investigated.

This study demonstrates that differences in results obtained from mitogen-stimulated culture of gravity sedimentation cells and cells recovered after sedimentation on Ficoll-Paque columns are due to
a) Differences in optimal mitogen concentrations for the 2 culture systems.
(b) Alterations in mononuclear cell sub-populations during isolation on Ficoll-Paque.

Po.17 T CELLS FROM BOVINE GAMMA GLOBULIN (BGG) TOLERANT MICE RETAIN THE ABILITY TO PROLIFERATE SPECIFICALLY AGAINST BGG IN VITRO

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Intravenous administration of soluble BGG reduces the ability of mice to produce anti-BGG antibody following immunisation. This tolerance has been attributed to a functional loss of BGG-specific T-helper cells. However, we have discovered that splenic T cells from BGG-tolerant mice can proliferate strongly to BGG in vitro. In fact, these T cells showed a specific response to BGG characteristics of a primed T cell population with a magnitude between that of primed and non-immune cells. Cells recovered from primary cultures of T cells from BGG-tolerised mice could be restimulated with BGG to give an anamnestic response. These results suggest that BCC-tolerance may not be due entirely to the deletion of BGG-specific T-helper cells.

Po.18 REGULATION OF PROLIFERATIVE RESPONSES TO OXAZALONE IN THE MOUSE

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Topical exposure of mice to 4-ethoxymethylene-2-phenyloxazole-5-one (oxazalone) at all contact sensitising concentrations results in a systemic depression of proliferative responses upon subsequent application of the same chemical. The observed reduction in the frequency of draining lymph node cells cycling in response to oxazalone in suppressed animals is attributable to a hapten non-specific population of T lymphocytes which can be demonstrated within 36 hours of initial exposure and which persists for up to 6 weeks.

The nature and mode of action of anti-proliferative lymphoid cells is currently being investigated and the model used to examine the relationship between primary T cell proliferation and the induction of contact sensitisation.

Po.19 KINETICS OF LYMPHOCYTE ACTIVATION

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PHA stimulated peripheral blood lymphocytes were examined at two-hourly intervals for changes in volume, the appearance of cell membrane receptors and nucleic acid synthesis. The time course was monitored by volume spectroscopy and cytofluorimetry using stochastic models to assess the percentages of cells responding in each system. The kinetics of appearance and changes in distribution of these activation events were considered in relation to possible restriction points in lymphocyte activation.

Monoclonal antibodies raised against early-activated T-cells were assessed during the time course and were compared with the known membrane markers of cell cycle progression.

Po.20 LYSOSTRIP EXPERIMENTS WITH HLA CLASS-II ANTIGENS

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We report results of lysostrip experiments designed to analyse the molecular and genetic relationship between different kinds of HLA class-II antigens. The purpose of lysostrip was to determine whether these antigens could be independently removed from the B-cell surface by incubation with specific antibody followed by incubation with Fab, fragments of rabbit antibodies against human IgG. Clearcut results have been found for DQ and DR antigens, which were removed independently indicating that they are located on distinct molecules, and supporting the concept of separateness of DQ and DR loci. A more complex situation was observed in the case of private DR, and supertypic DRw52 and DRw53 specificities. Different types of determinants were found, the one removed only by supertypic anti-DR sera, the second removed by private DR sera, and the third removed by private and supertypic DR sera as well. This may correspond to different beta chains present in DR molecule, coded at separate loci in DR region.

Po.21 MOUSE/HUMAN HETEROHYBRIDOMAS SECRETING ANTI-HLA-DR ANTIBODIES

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There is considerable scientific and clinical interest in the production of human monoclonal antibodies to HLA antigens. We have previously reported the production of oligoclonal human B-cell lines secreting antibodies which react preferentially with the HLA class II antigen DR5. We report here the production of mouse/human heterohybridomas from one of these cell lines. These have been cloned 2X at low dilutions (1/2 a cell/well) and continue to secrete IgM antibodies with specificities similar to those of the parent human B-cell line. The advantages of heterohybridomas for the production of monoclonal human antibodies to HLA antigens will be discussed.

Po.22 CLASS II MHC ANTIGEN (HLA-DR) EXPRESSION IN THE DEVELOPING SWEAT GLAND

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Class II MHC antigen (HLA-DR) is a normal constituent of the ductular lining cells of the sweat gland. In development, HLA-DR is first expressed on the developing bud of the growing gland as it extends down from the epidermis. The cells immediately behind the bud lose this staining. The antigen subsequently appears on the ductular lining cells and extends from the acrosyringium down the full length of the duct. The coils are negative. The appearance of the antigen in these two different situations suggests that it has a role both in embryogenesis and in the normal functioning of the mature gland.

Po.23 VARIATION OF CONSTITUTIVE MHC CLASS II ANTIGEN EXPRESSION IN RATS DETECTED BY AN ANTIBODY OF PARTICULAR HIGH AVIDITY

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MHC class II antigens are a major factor determining graft immunogenicity. Such antigens are thought to be regular structures on dendritic cells and macrophages but not or only rarely expressed on endothelial cells and definitely not on parenchymal cells of many rat organs. This basic supposition is challenged by studies of 9 organs of normal rats in 10 inbred strains of different allotypes with the commercial anti Ia Mabs OX6 (anti I-A) and OX17 (anti I-E) in comparison to our own cytotoxic anti I-E Mab 29A1. Other than OX6 and OX17, 29A1 is also available in a high titer version (29A1-HT) as culture supernatant, collected only in the first two weeks after careful recloning of hybridoma cells. Results of immunoperoxidase histology studies: (a) In the 6 non-RTI^{c} strains, OX6, OX17, 29AI and 29AI-HT showed identical reaction patterns, i.e. reactivity mainly with dendritic cells and macrophages in all tissues. (2) In contrast, in the 4 RTI^{c} strains, 29A1-HT showed additional reactions with capillary, venous and arterial endothelial cells in all tissues and also with parenchymal cells, e.g. pancreatic islet beta cells, hepatocytes and adrenal gland medullary cells. Conclusion: Constitutive I-E antigen expression in many cell types of rat organs is genetically variably determined. Suggestions: (1) This

finding appears to explain the higher immunogenicity of RTI^{c} grafts, expressed by their poorer survival, as suggested from pancreas islet grafting data. (2) Similar variations of MHC class II antigen expression appear to hold for humans of different HLA haplotypes as presently investigated.

Po.24 EXPRESSION OF CLASS II ANTIGENS ON HUMAN EOSINOPHILS

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Eosinophils from human peripheral blood were isolated by Percoll gradients ranging from 1.070 g/ml to 1.100 g/ml.

90-95% of pure eosinophils were obtained between 1.070 and 1.080 g/ml.

The presence of class II antigens on the surface of eosinophils were performed by immunofuorescence using a monoclonal anti-human DR (EDU, Dr. Vives, Barcelona) and a fluoresceinated goat F(ab')2 anti-mouse, and samples analyzed in a flow cytometer EPIC S-C (Coulter).

The expression of class II antigens was studied in non-treated eosinophils and in eosinophils cultured in the presence of recombinant human-gamma-interferon kindly provided by Dr. Adolf (Gentech).

In non-treated eosinophils, the maximal percentage of positive DR cells (30-40%) could be shown at the second day of culture. In the presence of different doses of interferon (10,50,100, and 500 IU) the percentage of positive cells seems to remain unaltered, but the maximum expression is reached earlier. Immunoprecipitation studies as well as different doses of gamma interferon are in course.

Po.25 EFFECT OF CYTOMEGALOVIRUS INFECTION ON THE EXPRESSION OF CLASS I HLA ANTIGENS IN VITRO

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The effect of cytomegalovirus (CMV) infection on the expression of Class I HLA antigens on human embryo lung fibroblasts was investigated. The level of HLA expression was quantified on fibroblast monolayers using scanning and

integrating microdensitometry in conjunction with a murine monoclonal antibody specific for Class I HLA directly conjugated to glucose oxidase. CMV infection was found to dramatically increase the level of HLA expression on fibroblasts, with peak expression occurring at 72 hours post infection. Similar enhancement of Class I HLA expression was seen using both laboratory passaged and clinical sources of CMV.

Po.26 **CLASS I EXPRESSION ON EK CELLS**

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We have studied the expression of MHC class 1 genes on EK cell lines CC1.2 and HD14 before and after differentiation in vitro. CC1.2 and HD14 are two EK cell lines independently derived from the inner cellmass of 129/Sv (H-2b) mouse embryos.

Total mRNA extracted from undifferentiated CC1.2 and HD14, and at various stages of differentiation, were analysed for the presence of class I transcripts using Northern blot and S1 nuclease protection analysis.

No Db transcripts were detected in mRNA from undifferentiated CC1.2 and HD14, 14 days differentiated CC1.2, 46 days differentiated CC1.2 and 32 days differentiated HD14 using S1 nuclease protection analysis.

In contrast, Northern blot analysis reveals the presence of low levels of H-2K transcripts in undifferentiated CC1.2 samples and, furthermore, that there is no significant increase in the levels of H-2 K mRNA after in vitro differentiation. These findings contrast with the data from F9 EC cells which will transcribe increased levels of class I mRNA after the in vitro differentiation to extraembryonic endoderm.

Po.27 **LEU 8 POSITIVE CELLS IN SKIN & BLOOD IN PATIENTS WITH CUTANEOUS LYMPHOMA**

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Leu 8, a marker for regulatory T cells positive in 75% of normal circulating T cells, has been found to be unexpectedly low in the cutaneous infiltrate of patients with cutaneous lymphoma.

We studied simultaneous skin biopsies and peripheral blood samples from 13 patients with stage 1 mycosis fungoides. Immunoperoxidase with Leu 8 marked 17% (5-30%) of cells in the infiltrate (helper: suppressor ratio 3:1) while FACS analysis of peripheral blood showed a slight reduction compared to age- and sex-matched controls (61.4 : 53.2).

The absence of Leu 8 positive cells in the dermal infiltrate cannot be explained by significant overall deficiency of circulating Leu 8 positive cells, but may be due either to selective sequestration of $T4^+$ Leu 8^- lymphocytes or possibly to loss of Leu 8 marker from T cells in the cutaneous infiltrate.

Po.28 **CYTOPLASMIC EXPRESSION OF T3 (CD3) IN NORMAL AND LEUKAEMIC T-CELLS**

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T3 (CD3) monoclonal antibodies detect different proportions of membrane CD3 (mCD3) on human thymocytes and this has led to their subdivision at the Boston Workshop into CD3a (UCHT1), CD3b (T10B9) and CD3c (OKT3) which are found on 60-70%, 40-50% and 20-30% of thymocyte membranes respectively. Co-capping, co-modulation and competitive binding experiments have shown that each of these CD3 subgroups represents separate determinants on the T3 complex. These subgroups of CD3 antibodies have been used to examine the membrane (mCD3) and cytoplasmic (cCD3) expression of CD3 in normal and malignant T-cells.

Cytoplasmic Cd3a and cCD3c were detected in >98% of normal thymocytes which lacked mCD3 and this cytoplasmic staining was seen in the perinuclear and Golgi areas. This cytoplasmic staining was not seen with CD3b (T10B9) which showed instead a cross-reactivity with intracellular

filaments which were not restricted to T-cells but could be found in other leukocytes, smooth muscle, endothelial and epithelial cells. This same filamentous reactivity was also found in primates (S.oedipus and M.rhesus) which lacked mCD3 on their leukocytes.

The expression of cCD3a and cCD3c was found in BrdU incorporating mCD3-TdT$^+$, CD7$^+$ thymic blasts which probably represent the earliest evidence of T-cell commitment. No cCD3$^+$ cells were detected among TdT$^+$ bone marrow cells. Only leukaemic T-cells (T-ALL) showed the same phenotype as these thymic blasts (cCD3$^+$, TdT$^+$) which was associated with TCR gene rearrangement. On the other hand no cCD3 was found in common ALL, including a case with aberrant TCR gene rearrangement, or in AML, including cases expressing one of the earliest T-cell antigens (CD7).

Transcription of CD3 (delta, epsilon or gamma) chains appears to be one of the earliest events in T-cell ontogeny and is detected by cCD3a and cCD3c. Insertion of the TCR-T3 complex into the membrane occurs later. A similar phenomenon is seen with IgM in B-cell development. The greater expression of mCD3a on thymocytes suggests either that it is inserted earlier into the membrane than CD3c or that the CD3c determinant is initially hidden and subsequently becomes exposed in thymocyte development.

Po.29 **MONOCLONAL ANTIBODY 60.3 SHOWS INCREASED REACTIVITY WITH CELLS FROM DOWN'S SYNDROME COMPARED WITH NORMAL**

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The monoclonal antibody (MoAB) 60.3 reacts with a complex cell surface antigen at least part of which is encoded by chromosome 21. This antigen is probably identical to human LFA-1ß which is involved in cellular immune reactions and homotypic cellular adhesions. We have investigated the reactivity of 60.3 with cells from subjects with Down's Syndrome (DS) (Trisomy 21), using fluorescence flow cytometric (FFC) analysis. EBV-transformed B-lymphoblastoid cell lines (LCL) from DS and normal subjects were treated with 60.3 or W6/32 which reacts with the HLA class I alpha chain encoded by chromosome 6, and stained with FITC conjugated rabbit anti-mouse Ig. FFC analysis of pairs of normal and DS cells at the same instrument settings revealed a significant increase in peak fluorescent signal for DS cells stained with 60.3 compared with normal. No such difference was found with cells stained with W6/32. Since no

difference was found in the percentage of 60.3 positive cells in DS and normal cells, we conclude that the results indicate an increased level of expresion on DS cells. probably due to gene dosage. Similar investigations were performed using cells with chromosome abnormalities other than DS, notably autosome and X chromosome deletions. These cells showed no increase in 60.3 reactivity over normal cells. It would appear that one result of Trisomy 21 is an increase in the expresion of LFA-1. To verify this, other LFA-1 MoAB have been tested. Some of these were also found to show a higher reactivity with DS cells than with normal.

Po.30 **IN VITRO GENERATION OF A CYTOTOXIC HUMAN MONOCLONAL ANTIBODY AGAINST HUMAN LYMPHATIC CELLS**

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Monoclonal antibodies (monabs) derived from mouse-mouse hybridomas are foreign proteins for a human recipient and patients in general develop anti mouse antibodies. Therefore the use of mouse monabs is limited and human monabs are needed for the in vivo treatment of patients. In order to find optimal conditions for the in vitro immunization of human B-lymphocytes for the production of human monabs, we added irradiated HLA different human lymphocytes to human splenocytes. After fusion with the mouse myeloma Ag8-653 the supernatants were screened using the microlymphocytotoxic assay and a hybridoma was found reactive against lymphatic cells of all persons tested, irrespective of their HLA antigens. The hybridoma Ha6D3 was cloned several times and has been stable now for more than a year and produces human IgM. Ha6D3 reacts against most B- and T-cell lines, but not against the non lymphoid cell lines HL60, U937, ML1, KG1 and K562. Biotinylated Ha6D3 made visible by an avidin peroxidase complex binds to the cell surface of most T- and B-lymphocytes of lymphnode and tonsil cryosections. Therefore Ha6D3 might be a candidate to replace xenogeneic antibodies for the treatment of graft versus host disease of bone marrow recipients or for the treatment of rejection crises after organ transplantation.

Po.31 THE DETERMINATION OF ABH ERYTHROCYTE ZYGOSITY BY AN ELISA-LIKE ESTIMATION OF ANTIGEN DENSITY

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Using routine agglutination techniques, the only means for assessing ABH genotypes requires extensive family typing. Measurements of plasma inactive-galactosaminyl transferase cross-reactive protein are related positively to the levels of H antigen, while radiolabelled reagents may give a direct estimate of erythrocyte antigen density. These latter two techniques, however, are time consuming and require elaborate preparation and equipment. In this report we describe a method utilising the relevant erythrocyte cells immobilised on the plastic surface of 96-well microtiter plates. After immobilisation, the cells were incubated with commercial anti-A or anti-B immunoglobulin preparations at the appropriate dilutions, followed by incubation with anti-human IgG/IgM conjugated to alkaline phosphatase, the developed enzyme activity being proportional to the antigen density. Alkaline phosphatase conjugated directly to the following lectins was also examined for its ability to detect blood group specific glycosides: Ulex europeus (anti-H), Helix pomatia (anti-A_1 and anti-A_2), Bandeiraea simplicifolia isolection B_4 (anti-B) and Dolichos biflorus (anti-A_1). Using this panel of immunoglobulin and lectin reagents, not all of which expressed effective binding in their conjugated form, distinct patterns of binding were observed for each genotype, allowing determination of zygosity in a short technically simple optical assay.

Po.32 MHC EXPRESSION & ANTIGEN PRESENTING PROPERTIES OF BRAIN ENDOTHELIUM

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Capillary endothelium was isolated from rat brain, cultured in monolayers and stimulated with recombinant rat IFN Expression of MHC class I and class II molecules was quantitated by an enzyme immunoassay using Ox18 (class I-specific), Ox6 (Ia-specific) and OX17 (Ie-specific) monoclonal antibodies. IFN gamma enhances class I expression to a plateau over the range 5-200 U/ML. Class II expression was undetectable on unstimulated cells, but was induced in a dose dependent manner over this range. Ia was markedly increased, but the induction of Ie, although significant, was marginal. The antigen presenting properties of these cells will also be discussed.

Po.33 SUBPOPULATION OF RAT B-LYMPHOCYTES OX-19, THE HOMOLOGUE OF MURINE LY-1

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OX-19 is a surface membrane molecule expressed by all rat T cells. Its homologue in mice (Ly-1) and humans (Leu-1/T1) is additionally expressed by a subpopulation of B cells which are thought to play a role in autoimmunity.

Using double immunofluorescence we detected a small subpopulation of normal rat B cells expressing OX-19. These had a similar distribution to mouse LY-1 cells; they were present in spleen and peritoneal cavity but absent in lymph nodes and Peyer's patches. They appeared early in ontogeny and reached adults level by 4 weeks of age. Their numbers were not influenced by immunization with T-dependent or T-independent antigens. The functional significance of this B cell subpopulation will be discussed.

Po.34 ANTIGEN DETERMINANTS FOUND ON GUINEA PIG LEUKOCYTES

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Two mouse anti guinea pig monoclonal antibodies designated MSgp 1 and MSgp 2 were produced from a fusion between myeloma cells and mouse splenocytes immunized with guinea pig thymocytes and B-cells respectively.

The MSgp 1 determinant is expressed by a subset of small thymocytes and lymph node T-cells as determined by Forward Light Scatter by FACS analysis. The determinant is modulated by antigen in vivo and the MSgp 1 antibody will prevent MHC Class II driven proliferation in vitro, which may indicate that the MSgp 1 determinant is involved with helper cell function.

MSgp 2 reacts with an antigen present on the majority of lymphocytes and is expressed on Langerhans cells as defined by cell morphology, expression of Class II and the presence of Birbeck granules in positive cells. The tissue distribution of MSgp 2 antigen on lymphocytes of the lymph node could indicate a role of this determinant in cell migration.

Po.35 FLOW CYTOMETRIC ANALYSIS OF HUMAN LUNG LYMPHOCYTE POPULATIONS

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The problem of lymphocyte contamination with human red cells is well recognised in flow cytometry. The partial overlap of the lymphocyte and erythrocyte populations causes inaccuracy in total lymphocyte enumeration as well as percentage counts in fluorescent monoclonal antibody studies.

In studying lymphocyte populations in tissue sites and body fluids, as distict from blood, it is not always appropriate to use Ficoll-Hypaque density separation techniques to purify the lymphocytes and remove erythrocytes prior to FACS analysis. Lymphocyte numbers are often too small to allow such a purification step and there are also problems of contamination with other cell types of similar density.

Hypotonic lysis methods do not completely overcome this problem because not all the red cells are lysed. The results are often variable within the same individual and between different individuals, especially in disease when red cell fragility may be altered.

We have developed an improved method of simultaneously staining the cells for surface antigen and DNA and triggering the FACS on DNA fluorescence. Thus, non-nucleated cells are excluded from the analysis and accurate gating on the lymphocyte population is facilitated.

This method and its application to the analysis of lung lymphocyte populations will be described.

We gratefully acknowledge the British Lung Foundation and the Clinical Research Committee of the National Heart and Chest Hospitals for their support.

Po.36 CHARACTERISATION OF SPERM ANTIGENS REACTING WITH HUMAN ANTISPERM ANTIBODIES

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Antibodies reacting with human spermatozoa have been detected by various immunological techniques in the sera of subfertile men. Different patterns of sperm agglutination are observed with different sera either head to head, tail to tail, or tail-tip to tail-tip. In the present study immunoblotting techniques were used to characterise the reactivity of solubilised sperm proteins with serum samples exhibiting different modes of sperm agglutination. The results showed that although antisperm antibodies bind to discrete and specific sperm-associated antigens, there is no substantial difference between the antigenic patterns observed with antibodies producing different types of sperm agglutination.

Po.37 EXPRESSION AND MODULATION OF CELL SURFACE ANTIGENS ON HUMAN NEONATAL & ADULT MONOCYTES

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Differences between newborns and adults in the antigen-specific plaque forming cell response could be ascribed to differences in antigen presenting capacities of the respective monocytes (1). This suggested us to study expression of cell surface antigens of human neonatal and adult monocytes by use of various monoclonal antibodies to membrane proteins including MHC antigens. No difference was observed in expression of LeuM3, OKM1 and OKM5 with regard to both the percentage of positive cells and the density of the antigenic determinants. Neonatal and adult monocytes expressed class I MHC antigens in similar density. Expression of class II MHC antigens was studied using monoclonal antibodies against HLA-DP, -DQ and -DR. Slight differences in expresion of all three antigens were found on adult and neonatal monocytes with a tendency of weaker expression on cells of the neonate.

Modulation of HLA-DR expression by gamma-interferon was observed both on neonatal and on adult monocytes to the same extent.

Possible relevance of these findings to differences in monocyte function will be discussed.

(1) Van Tol et al, 1984. J. Immunol. 134: 1902-1908.

CLINICAL IMMUNOLOGY

Po.38 NON-CYTOTOXIC MATERNAL ALLOANTIBODY RESPONSES DURING EARLY PREGNANCY DETECTED BY A CELLULAR ELISA

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Using a cellular ELISA (CELISA), we have examined sera from nulliparous women and women in the first trimester of a first or subsequent pregnancy for the presence of IgG antibodies which bind to peripheral blood lymphocytes from unrelated donors. Maternal antibody activity was found in sera from 1/13 nulliparae, 18/37 primigravidae and 8/12 multigravidae. Cytotoxic antibody was present only in 3/12 multigravid sera. Absorption with packed, pooled platelets did not remove antibody activity from primigravid sera; unabsorbed sera, however, bound equally well to T and B lymphocytes. These data suggest that the antibody detected by CELISA is not directed to any of the classical HLA antigen series.

Po.39 INTERLEUKIN-1 & PROSTAGLANDINS IN ULCERATIVE COLITIS

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Ulcerative colitis (U.C.) is a chronic inflammatory disease associated with disturbed immune function and an acute phase response. Mononuclear cell release of interleukin-1 (IL-1) and prostaglandins, PGE2 and 6KF1a, was examined in U.C. patients (n=23) and healthy subjects (H.S. n=15). Mononuclear cells were cultured for 24 h and supernatants assayed for IL-1, using the co-mitogenic mouse thymocyte proliferation assay, and for prostaglandins, by radio-immunoassay.

Spontaneous and lipopolysaccharide-induced IL-1 and 6KF1a, but not PGE2, levels were higher in U.C. than H.S. These changes were unrelated to monocyte number of disease activity (Truelove score). IL-1 and prostaglandin production were not correlated.

These results provide evidence for elevated monocyte function in U.C., though the relevance of this to the pathogenesis of the disease remains unclear.

Po.40 ABNORMAL IgG3 REGULATION IN PRIMARY BILIARY CIRRHOSIS(PBC)?

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We are examining the origin of the raised serum IgG3 in PBC patients. PBC blood lymphocytes in culture spontaneously synthesise a higher percentage IgG3/IgG than do controls, as determined by ELISA. This correlated with their serum IgG3 and suggests that there is increased synthesis of this isotype, rather than reduced clearance. With pokeweed mitogen the proportion of IgG3/IgG synthesised by normal (and most PBC) lymphocytes increases and the difference between PBC and controls becomes less marked. The kappa/lambda light chain ratio of the IgG3 shows no evidence for clonally restricted synthesis of IgG3 in PBC.

Po.41 IMMUNE STATUS IN UVEITIS: AN INVESTIGATION OF IMMUNOCOMPETENCE & SPECIFIC RESPONSIVENESS OF UVEITIS PATIENTS IN DISEASE & THERAPY

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Inflammatory disease of the uveal tract (uveitis) is an important cause of ocular disability and visual loss. It has been postulated that uveitis may have an autoimmune aetiology due to observed alteration in the normal frequency of certain lymphoid cell populations ie. T lymphocytes and the suppressor cell subpopulation in particular (Nussenblatt et al, 1983. Am. J. Ophthalm. 95, 614). The aim of the present study was to determine the general immune status and specific immune responsiveness of patients with uveitis and related disorders (e.g. retinitis pigmentosa) to retinal S-antigen in particular. Humoral immunity was assessed by

total immunoglobulin determinations and ELISA measurement of antibodies to S-antigen in both serum and the supernatant from pokeweed mitogen (PWM) stimulated lymphocytes. Cellular immunity was assessed by lymphocyte transformation to non-specific lectin stimulation (PHA-P, Con A) specific common antigens, PPD (purified protein derivative of tubercule bacillus) and SK/SD (streptokinase/streptodornase enzymes) and to retinal S-antigen itself.

A total of 30 uveitis patients and 14 retinitis pigmentosa patients were investigated in conjunction with age and sex-matched controls from an accredited donor population (with no history of uveal/ophthalmic disorders). The results are discussed in relation to the heterogeneity of the clinical classification within the uveitis study group and their respective therapies.

FIRST FLOOR FOYER

42. PROGNOSTIC VALUE OF IgD LEVELS IN ANTI-HIV POSITIVE HAEMOPHILIACS

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An important AIDS-related problem which needs to be resolved is the identification of laboratory markers which can predict disease progression in anti-HIV positive patients. Haemophiliacs form a unique study group because in many cases serial serum samples are available from the period before seroconversion, through seropositivity, persistent generalized lymphadenopathy (PGL) and AIDS-related complex (ARC), to AIDS itself. The purpose of this study was to assess the prognostic value of IgD levels in seropositive patients. Initial assessment shows that anti-HIV positive haemophiliacs have increased levels of IgD (geometric mean 15.33 $\pm$ 1.04 S.E.M., n=68) compared with anti-HIV negative patients (3.66 x 1.08, n=25) and normal laboratory personnel (0.74 $\pm$ 0.47, n=12). In anti-HIV positive haemophiliacs with PGL, much higher IgD levels were found (59.14 $\pm$ 1.19, n=10). In 2 seropositive patients who subsequently developed AIDS there was a progressive decline in IgD levels. The patients with high levels of IgD are now regularly followed for the decrease of IgD levels, T4/T8 subsets and other clinical signs. IgD levels appear to have prognostic significance in seropositive haemophiliacs.

Po.43 LYMPHOCYTE DYSFUNCTION IN CHRONIC SUPPURATIVE LUNG DISEASE

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We report three patients with progressive chronic suppurative lung disease of childhood onset associated with cellular immune deficiency. The patients were anergic to a range of delayed type hypersensitivity skin test antigens. Peripheral blood lymphocytes were present in normal numbers, but OKT4a positive lymphocytes were decreased. In vitro proliferative responses of the patients' lymphocytes to mitogenic stimulation were impaired. Immunoglobulin-secreting plaque-forming cell numbers following stimulation with PWM were reduced in two patients. Serum IgG was normal or elevated, but serum IgG2 was deficient in two patients tested. The cellular immune abnormalities detected in these patients have not been described previously in association with bronchiectasis.

Po.44 INFLUENCE OF VITAMIN C & ZINC ON IMMUNE STATUS IN CROHN'S DISEASE

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The immune status of 29 Crohn's disease patients supplemented with Vitamin C, zinc or placebo was studied. Peripheral blood mononuclear cells were isolated. Monocyte adherence and latex ingestion were enumerated. Monoclonal antibodies (OKM1, UCHT1, Dako T4, RFT8 and Leu 11b) were used for phenotyping. Lymphocyte function was assessed by phytohaemagglutinin stimulation and tritiated thymidine incorporation.

Plasma Vitamin C and zinc concentrations rose in the respective patient groups ($p<0.05$). PHA stimuation improved only in the Vitamin C group at all mitogen doses ($p<0.05$) and in the zinc group at the highest dose only ($p<0.05$). No significant change occurred in the other parameters.

In conclusion, although Vitamin C appeared to improve lymphocyte function, zinc did not consistently do so.

Po.45 **EFFECT OF URAEMIA ON PRODUCTION OF IMMUNOGLOBULIN IN VITRO**

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We investigated the effect of uraemia on production of immunoglobulin (Ig) in vitro by enumerating both cells spontaneously secreting Ig and after stimulation with either pokeweed mitogen (PWM, T-dependent), S.aureus (SAC, relatively T-independent) or Epstein-Barr-Virus (EBV, T-independent).

The spontaneous plaque forming cell response (PFC) of uraemic peripheral blood mononuclear cells (PBMC) was significantly lower than that obtained by control cells and the same was observed when SAC was used. In contrast, PWM and EBV induced PFC responses were not different from those obtained in controls. Analysis of T and B lymphocyte numbers showed that these were within the normal range.

It is known that B cell proliferation in response to SAC is T cell independent while full differentiation of the B cell depends on T cell help which is probably supplied by background stimulation of helper T cells. It is concluded that a defect on T lymphocyte function may account for the reduced spontaneous and SAC induced production of Ig by uraemic PBMC.

Po.46 **IN VITRO EFFECT OF STEROIDS ON CONTROL & URAEMIC LYMPHOCYTE RESPONSES**

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The in vitro effect of steroids on both the proliferative and plaque forming cell (PFC) responses of control and uraemic peripheral blood mononuclear cells (PBMC) were examined.

The proliferative responses to a T cell mitogen (PHA) were significantly lower in uraemics but the immunosuppressive effect of steroids was not found to be stronger in uraemic cultures as previously suggested.

Pharmacological concentrations of steroids are known to inhibit selectively suppressor T cell function. S.aureus (SAC) induced PFC responses of control PBMC were enhanced by the addition of steroid indicating that the SAC system is influenced by suppressor T cells. In contrast, the low SAC induced PFC responses of uraemic PBMC were not improved by the addition of steroid.

Our results suggest that there is no difference in steroid sensitivity between control and uraemic T lymphocytes and also that increased suppressor cell activity does not account for the low SAC induced PFC responses in uraemia.

Po.47 **SPECIFICITY OF HUMAN ANTI-CAPTOPRIL & ANTI-PENICILLIN ANTIBODIES CHARACTERISED BY ELISA**

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IgG anti-captopril (CP) activity was detected by ELISA in sera from 2/45 patients receiving the drug (25-75 mg/day). Only one of five patients who experienced a drug-associated skin rash was antibody positive. IgG anti-CP activity was specific for disulphide-conjugated metabolites of CP as defined by ELISA inhibition.

Serum IgG anti-penicillin antibody was detected in 3/53 and IgE anti-penicillin in 1/53 patients with suspected allergy to penicillin. IgG and IgE activities were directed against both benzylpencilloyl (BPO) and ampicilloyl (AMP)-HSA. The specificity of IgG and IgE anti-BPO and anti-AMP activities for both parent drugs and their major metabolites was defined by ELISA inhibition.

Po.48 **PHENOTYPIC STUDIES ON THE LYMPHOCYTES & MONOCYTES INFILTRATING TUBERCULIN SKIN TEST SITES IN HAEMOPHILIA A PATIENTS, WITH & WITHOUT ANTIBODY TO HTLV3 VIRUS**

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6/13 patients showed a positive skin test to PPD: the proportion of the dermis occupied by focal infiltrate was similar in the HTLV3-antibody negative (mean 9.86%) and 10 positive reactors (mean 9.31%). The T4:T8 ratio in the diffuse dermal infiltrate was lower in the anti-HTLV3 positive patients than in negative reactors, but neither group had a T4 lymphopenia. The proportion of lymphocytes

bearing IL2- and transferrin-receptors was only slightly lower in the HTLV3 antibody positive patients than in the negatives. These results do not provide any substantial evidence of impairment of Type IV effector mechanisms in Haemophilia A patients with normal T4 counts in the peripheral blood, even when anti-HTLV3 positive.

IMMUNODEFICIENCY

Po.49 MEASUREMENT OF ANTIBODIES TO CANDIDA ALBICANS AS A SCREENING TEST FOR HUMORAL IMMUNODEFICIENCY

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Some patients have a normal or near normal concentration of serum immunoglobulins yet fail to produce specific antibodies to infecting organisms. In screening for defects in humoral immunity, as well as measuring serum immunoglobulin it is therefore important to have some measure of antibody production e.g. immunising with a test antigen and measuring the antibody response some days later. However, this delay may be inconvenient and requires a second blood sample. An alternative is to test whether the patient can make antibody to a widespread commensal organism to which he/she must have been repeatedly exposed. The measurement of antibody to E.coli by haemagglutination (1) has been used for this purpose.

We report here that an equally good and simpler alternative is to measure antibodies to the commensal Candida albicans by immunofluorescence. Using a polyvalent conjugate, 114/114 blood donors had antibody titres >8 to C.albicans; similar responses were noted in 20 children (aged 6 months - 16 years) without recurrent infections. In contrast, anti-candida responses were low or absent as expected in patients with hypogammaglobulinaemia but also in some patients with selective IgA deficiency and other immunodeficiency diseases. Further analysis of the anti-candida response revealed it to be predominantly of the IgG class.

(1) Webster ADB, Efter T, Asherson GL. Brit. Med. J. 3, 16-18 (1974).

Po.50 CYTOTOXIC & NONCYTOTOXIC $CD3^-$ NK & $CD3^+$ T CLONES DERIVED FROM A PATIENT WITH SEVERE COMBINED IMMUNODEFICIENCY (SCID)

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Patients with severe combined immunodeficiency (SCID) usually have very low levels of circulating T cells, B cells and lack mitogenic responses in vitro and have depressed natural killer (NK) cell activity. Little is known about functional and biochemical properties of T cells and NK cells in SCID compared to healthy individuals. In vitro expansion of cell lines facilitates such studies. Using a culture system efficient for the rapid expansion of normal T cells, both $CD3^+$ and $CD3^-$ NK cell clones were expanded from a patient's peripheral blood lymphocytes (pbl). The NK clones were $CD2^+$ $CD3^-$ $CD16^+$ (CD16 is IgG-Fc-Rec) like normal NK cells, lysed a broad range of target cells including fresh tumor cells, and exert antibody dependent cellular cytotoxicity (ADCC). The $CD3^+$ clones were noncytotoxic and most of them expressed the CD4 antigen. Two clones were $CD3^+$ $CD4^+$ $CD8^+$ and three clones were $CD3^+$ $CD4^-$ $CD8^-$. Taken together, some clones resembled normal T cells, also found in pbl of healthy individuals, but others have different phenotypical and functional (cytotoxic) properties.

Po.51 PHENOTYPE AND FUNCTION OF B CELLS IN MHC DEFICIENCY SYNDROME

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We have investigated mononuclear cells of a family with two siblings with combined immunodeficiency and defective expression of MHC antigens on mononuclear cells (W.Kuis et al, BMT in Europe, Vol. 2, 1981, 201-208). The siblings aged 5 (male) and 1 (female) years respectively were born from healthy consanguinous parents. FACS analysis showed strongly reduced expression of class I antigen on T cells and monocytes and to a lesser degree also on B cells. Class II antigens (HLA-DR, -DP, -DQ), although early in life to some extent detectable on B lymphocytes and on mitogen

activated T cells became completely absent on all cells (whether resting or in vitro activated). Monocytes consistently were negative for class II expression even after in vitro treatment with gamma-interferon. The maturational stage of B cells was assessed by IgM/IgD and FMC7 expression. Patient's B cells showed, like neonatal B cells, a high sIgM/sIgD ratio and therefore appear immature. However, patient's B cells express 20-40% FMC7 which contrasts the results with normal neonatal B cells which express 100% FCM7. Because of the defective expression of MHC antigens, B cell activation dependent upon MHC interactions is impaired in both patients. By using polyclonal activators such as PWM and SAC, IgM secreting plasma cells can be induced in vitro, but IgG and IgA plasma cells are lacking. As compared to cultures of control donors, supernatants of cultures of patient's cells did not contain antibodies to polysaccharide antigens, an observation which seems to contrast the findings reported in literature that these patients can produce antibodies following Pneumovax immunization.

Po.52 **EXPRESSION OF MAJOR HISTOCOMPATIBILITY COMPLEX ANTIGENS IN TISSUE IN PATIENTS WITH "BARE LYMPHOCYTE" SYNDROME**

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We previously reported on the absence of HLA-class I and II antigens on epithelial cells in the thymus biopsy of patients with "bare lymphocyte" syndrome, which patients had a combined immunodeficiency and a defective expression of HLA antigen on blood mononuclear cells (BSI Autumn meeting 1983). We subsequently studied HLA expression in skin from one of these patients and from his younger sister, and in placenta obtained at delivery of this younger sister. Both patients lacked HLA class II on blood mononuclear cells and had a reduced expression of HLA class I on these cells (see accompanying presentation by Rijkers et al. Po.51).

Placenta: at the maternal side there was HLA class I and class II antigen expression as in normal placenta, but endothelial cells and mesenchymal stroma at the fetal side in the villi lacked class II expression (DR and DQ). At this location there was only a faint expression of HLA class I. This finding was confirmed by the absence of HLA expression on cells in umbilical cord blood. This suggests that it may be possible to document "bare lymphocyte" syndrome by immunohistochemistry on chorionic-villus biopsy early in pregnancy.

Skin: biopsies from both patients showed a normal density of Langerhans cells in the epidermis (e.g. assessed by their positivity for CD1, OKT6). Class I antigen expression was variably reduced in keratinocytes, endothelial cells and Langerhans cells. Class II expression (DR, DP and DQ) was absent on Langerhans cells, and some of the antibodies gave a faint staining of blood vessels. This indicates that the defective HLA expression in these patients is not restricted to blood mononuclear cells, but also concerns other tissues. For instance, concerning cells involved in antigen presentation, the defect in HLA class II expression is not restricted to blood monocytes, but applies also for Langerhans cells in skin.

Po.53 **CONGENITAL THYMIC APLASIA WITH MINIMAL IMMUNE DEFICIENCY**

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A.D. (DoB 20/11/84), a white male child, underwent elective surgery for closure of atrial and ventricular septal defects at 7 months of age. At operation no thymus gland or tissue was identified. Immunological investigation showed normal numbers of circulating lymphocytes. Proliferative responses of peripheral blood mononuclear cells to mitogenic stimulation were normal. IgG, IgM & IgA secreting plaque forming cels were detected in levels comparable to those of a healthy adult. NK activity was normal. Serum thymic hormone level, however, was markedly reduced. Clinically the child is well and has been free from infection since the operation. This case would appear to represent congenital thymic aplasia with minimal immune deficiency.

IMMUNOPATHOLOGY

Po.54 **SUPPRESSION OF IgE BY HYPERIMMUNE SERUM IN RATS**

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Antigen-specific IgE suppression, accompanied by IgG enhancement was obtained when a small volume of EA-hyperimmune serum (0.1 ml) was administered to adult rats, at the same time as immunisation. The effects seen declined with increasing volumes of serum.

It is considered that, when the small volume of passive antibody was given, an immune complex formed between it and the immunising antigen, and that unfavourable conditions of antibody excess were introduced when the volume of administered serum was increased.

Immunisation with preformed complexes and adjuvant, or treatment with complexes after immunisation, selectively suppressed IgE, whereas pre-treatment did not.

Such evidence suggests IgE responses can be regulated by immune complexes.

Po.55 **DETECTION OF SPECIFIC IgG AND SPECIFIC IgG SUBCLASS ANTIBODIES TO ACID ANHYDRIDE-HUMAN ALBUMIN CONJUGATES**

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Seven patients with asthma due to tetrachlorophthalic anhydride, an epoxy resin hardening agent, had specific IgE antibody to a tetrachlorophthalic anhydride-human serum albumin conjugate (TCPA-HSA) (1). We have developed ELISAs, using monoclonal antibodies to IgG (all subclasses), IgG1 and IgG4, to quantify specific IgG and specific IgG subclass antibodies to acid anhydride-HSA conjugates.

Results show that these seven patients, in addition to specific IgE, have specific IgG, IgG1 and IgG4 antibodies to TCPA-HSA. These antibodies were not detected in unexposed individuals. Specificity studies demonstrated that the assay system was specific for TCPA-HSA; neither unconjugated HSA, nor an unrelated hapten-HSA conjugate inhibited the assay.

(1) Howe W, Venables KM, Topping MD et al. J. Allergy Clin. Immunol. 1983; 71, 5-11

Po.56 **INVERSE CORRELATION BETWEEN INFLAMMATION, TOTAL SERUM IgA & HAEMOGLOBIN IN ANKYLOSING SPONDYLITIS**

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Erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), total serum IgA and haemoglobin (Hb) were measured in 135 ankylosing spondylitis (AS) patients (New York Criteria) during different phases of disease activity in an endeavour to determine any association between inflammation, total serum IgA and haematological status.

The mean ESR and CRP in patients with a Hb up to 12 G/dl was 56 mm/hr and 57 µg/ml respectively compared to a level of 15 mm/hr ($p<0.001$) and 11 µG/ml ($p<0.001$) in patients with a Hb of 14 G/dl and above and these differences were statistically significant.

Patients with intermediate Hb levels had correspondingly intermediate values for inflammatory parameters.

Low Hb levels were also associated with increased total serum IgA in all groups of AS patients having different degrees of disease activity as defined by biochemical criteria.

The mechanism for this negative exponential association remains unclear but it would appear that AS resembles to some extent another inflammatory condition, namely rheumatoid arthritis where a clear association between low Hb and inflammation has been recognised for some time.

Po.57 **EXPRESSION OF CLASS II ANTIGENS & mRNA IN JOINT CELLS IN RHEUMATOID ARTHRITIS**

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Excessive expression of HLA Class II antigens on target tissues is a marked feature of many autoimmune diseases including Rheumatoid Arthritis (RA). We have compared the class II protein expression and the mRNA levels for the various subclasses (DR, DP and DQ). The levels of mRNA were high, assessed by Northern and slot blot analyses. Compared with EBV transformed cell lines the proportion of class II mRNA was often higher in RA joint cells. The joint cell populations were placed in culture to determine the duration of expression, with or without various signals such as IFN gamma and IL-2. In certain individual class II mRNA levels persisted in culture, and were modulated by IFN gamma and IL-2.

Po.58 **INTERLEUKIN 2 (IL-2) DEPENDENT T-CELL CLONES FROM SYNOVIAL FLUID OF A PATIENT WITH RHEUMATOID ARTHRITIS (RA)**

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RA synovial fluid contains activated T-cells. These T-cells cannot be maintained for very long on IL-2 alone without

restimulation. Antibodies to the T3 molecule on T-cells (OKT3) allow the long term expansion, culture of these T-cells.

Synovial fluid cells from a patient with RA, containing 8.4% IL-2 receptor positive cells, were cultured with IL-2. 10 days later the culture was stimulated by OKT3. When cloned the cells were over 85% T cells. These cells were cloned with IL-2, OKT3 and autologous feeders and maintained on IL-2, OKT3 and either autologous or DR-matched feeders.

The ensuing panel of clones is being characterised. All are $T3^+$ $T11^+$ $T4^+$ $T8^-$. All express the 4B4 marker of "helper inducers". None express markers of suppressor inducers (2H4, TQ1, Leu8). An interesting aspect, not previously described, is the high expression of HLA-DQ. One clone expresses 5 fold more DQ than DR, a striking reversal of the usual ratio. Production of lymphokines is being assessed by bioassay and mRNA assays (for IL-2, interferon, lymphotoxin and tumour necrosis factor) No $T8^+$ cells grew out using this procedure, despite being present in the fluid originally. We are checking, by dual fluorescence to see whether any T8 cells express IL-2 receptors in vivo.

Po.59 IMMUNOGLOBULIN EFFECTS OF ANTI-CLASS II HLA ALLOANTIBODY INJECTIONS IN RHEUMATOID ARTHRITIS

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Placenta-eluted gammaglobulins (PEGG) have been eluted at acid pH from large pools of blood-free human placental tissue and shown to contain anti-class II HLA allo-antibodies. They were given intravenously to patients with severe classical Rheumatoid Arthritis. About 60% of the 34 treated patients were clinically improved as assessed by several subjective and objective parameters. In view of the role of class II HLA antigens in autoimmune processes we followed up different immunological parameters in order to relate possible biological effects with the action of anti-class II HLA alloantibodies. Using indirect immunofluorescence and quantitative flow cytometric methods we were unable to observe any drastic modulation of class II HLA antigens on the surface of blood mononuclear cells, except a slight and transient increase of monomorphic class II HLA determinants on B lymphocytes. We have looked for a possible anti-idiotypic immunisation against anti-class II HLA alloantibodies and found in certain patients a rise in anti-F(ab')2 antibodies of which the specificity is being investigated.

Po.60 INVOLVEMENT OF MACROPHAGE-LIKE CELLS IN THE RHEUMATOID PANNUS

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Frozen sections of pannus tissue taken from the joints of patients with rheumatoid arthritis have been investigated using immunohistological methods to determine the distribution of subsets of macrophage-like cells in this area. A panel of monoclonal antibodies including reagents specific in normal tissue for interdigitating cells (RFD1), macrophages (RFD7), epithelioid cells (RFD9), monocytes (UCHM1, kindly provided by Dr. P. Beverley) and osteoclasts (2C36, kindly provided by Dr. M. Horton) were used.

It was discovered that 80% of the lining cells and a majority of macrophage-like cells of the stroma express the unusual phenotype RFD1+, RFD7+, UCHM1+. Cells with a typical "dendritic cell" phenotype (RFD1+, RFD7-) were only present in the perivascular infiltrates while "classic" macrophages (RFD7+, RFD1-) were the cells accumulating at the cartilage junction. No significant numbers of RFD9+ epithelioid cells were seen. 2C36+ osteoclasts were present in small numbers distributed throughout the stroma but did not appear involved in areas of cartilage degradation. It is concluded that distinct inflammatory reaction occurs in the pannus area and that classic activated macrophages may be involved in cartilage degradation.

Po.61 ANTIBODIES TO PROTEUS SPECIES IN RHEUMATOID ARTHRITIS

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Antibodies to Proteus species were measured in sera obtained from 34 rheumatoid arthritis (RA) patients treated with gold and 18 healthy controls, using a double antibody ELISA method, with whole bacteria as the solid phase antigen.

Elevated antibody levels (O.D.x 10^{-2} units) were found in 14 active (CRP> 10 µg/ml; RF> 30 I.U./ml) RA patients: 13.8 + 1.7 (mean + SE) when compared to 18 healthy control subjects: 7.7 + 0.6 ($p<0.001$). (CRP = C-reactive protein; RF = rheumatoid factor).

Increased levels were also found in 12 probably active (Either CRP> 10 µg/ml or RF> 30 I.U./ml) RA patients: 11.6 ± 0.7 when compared to the same 18 control subjects ($p<0.001$).

Similarly, 8 inactive (CRP<10 µg/ml; RF< 30 I.U./ml) RA patients were found to have higher levels: 10.4 ± 1.3 than the 18 controls ($p<0.05$).

However the differences between the 3 disease groups were not statistically significant.

The role of Proteus microorganisms in RA merits further study.

Po.62 **THE DEPRESSED AUTOLOGOUS MIXED LYMPHOCYTE REACTION (AMLR) IN RHEUMATOID ARTHRITIS (RA) IS DUE TO A T CELL ABNORMALITY**

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The AMLR in RA has been shown to be depressed but the mechanism for this abnormality is not known. We have generated EBV cell-lines from HLA-DR identical RA patients and controls and used them as stimulator cells in the AMLR. The RA AMLR is still depressed. However, analysis of the intensity of HLA-DR expression by the RA and control EBV cells is identical. Thus the deficient AMLR cannot be due to defective DR expression by the RA EBV cells. This was confirmed by the fact that RA EBV cells acted as normal stimulators of an allogeneic MLR (aMLR). Furthermore, RA T cells responded in an aMLR to control EBV cells (46082 ± 22719 dpm) to the same desgree as their response to allogeneic RA EBV cells (60068 ± 14013 dpm). Thus, the defect in the AMLR in RA resides predominantly in the inability of rheumatoid T cells to recognise and/or respond to autologous stimulating antigens.

Po.63 **EXPANSION & CLONING OF T LYMPHOCYTES INFILTRATING THE THYROID GLAND FROM A PATIENT WITH HASHIMOTO'S DISEASE**

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T lymphocytes infiltrating the thyroid of an Hashimoto's patient were isolated and expanded for one week in presence of rIL-2, then cloned in presence of OK T3. 89 clones were thus obtained and 33% of the clones so far analyzed had this

phenotype CD3-CD8 positive and CD4-Leu11 negative. This is a different pattern from a panel of Graves' clones previously prepared. We studied the release of gamma interferon in the supernatant of these clones: range 2-520 U/ml, mean 126 ± 145, as such a lymphokine has the major role in inducing class II expression on thyrocytes. The release of other lymphokines and the functional analysis of these clones are under current study.

Po.64 **MIXED LEUCOCYTE REACTIONS (MLR) & SPECIFICITY OF UNCLONED THYROID LYMPHOCYTES IN THYROID DISEASES**

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MLR stimulation by various antigen presenting cells as well as the specificity of expanded intrathyroidal T lymphocytes were studied in patients with Grave's disease (GD) and compared to results obtained in patients with non-toxic goitre (NTG). For allogenic MLR stimulation HLA-DR+ thyrocytes, thyroid tissue macrophages and monocytes had similar activities in both diseases. This confirms the antigen presenting capacity of thyrocytes. In autologous MLR the stimulatory activity of thyrocytes was higher in GD than in NTG. Intrathyroidal T lymphocytes from GD and NTG patients expanded equally well with ORT3 and recombinant IL2. However, only in GD the uncloned T cell line proliferated specifically in response to autologous thyrocytes, suggesting autoantigen recognition in GD, but not in NTG.

Po.65 **HLA CLASS II SUBREGION EXPRESSION BY THYROCYTES IS ASSOCIATED WITH THE OCCURRENCE OF CIRCULATING THYROID AUTOANTIBODIES**

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HLA class II expression by thyroid epithelial cells (thyrocytes) is postulated to play an important role in the pathogenesis of thyroid autoimmunity. As one test of this hypothesis, we have investigated whether a relationship exists between the occurrence of such inappropriate Class II expression and circulating thyroid autoantibodies in 146 individual patients of various diagnoses from whom thyroid tissue was available. Thyrocyte Class II expression was found to be significantly associated with the occurrence of

autoantibodies to thyroid microsomal antigen (TMAb) and with autoantibodies to thyroglobulin (TgAb). Furthermore, in Graves' disease patients the most significant associations occurred between TMAb and thyrocyte expression of HLA-DR, and between TgAb and HLA-DQ.

These findings are consistent with the hypothesis that Class II^+ thyrocytes can effectively present autoantigens and thus stimulate autoimmune events such as autoantibody production. They further suggest that different HLA-D subregion products expressed by thyrocytes are dominant in stimulating responses to different thyroid autoantigens.

Po.66 **AUTOIMMUNE T-CELLS PRODUCE TUMOUR NECROSIS FACTOR**

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TNF and the related lymphocyte product lymphotoxin display multifunctional properties in vivo and in vitro. We have evidence which suggests cloned T-cells from Rheumatoid Arthritis and Hashimoto's patients may produce TNF.

Supernatants from these clones are positive for TNF in the L-929 assay. To eliminate the contribution of feeders, clones were stimulated with anti-CD3 conjugated to Sepharose and IL2 before mRNA was extracted. On Northern analysis the TNF probe hybridised to an 18S message which corresponds to the TNF message found in stimulated HL60 cells. Analysis of 14 other clones by slot blotting also revealed the presence of TNF mRNA. We do not know as yet whether it is normal for T-cells to produce TNF or the relevance of these findings to the autoimmune state.

Po.67 **LYMPHOCYTE Fc GAMMA-RECEPTOR BLOCKING IN ANTIBODIES IN INTRAVENOUS GAMMAGLOBULIN PREPARATIONS**

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Intravenous gammaglobulin (IV-IgG) therapy has been used in recent years to treat various types of autoimmmune disease but its precise mode of action remains unclear. It has been suggested that anti-lymphocyte antibodies, and in particular, lymphocyte Fc gamma R-receptor (Fc gamma R) blocking antibodies may play a role in the "down regulation"

of autoantibody synthesis which is known to occur following IV-IgG therapy. Such non-cytotoxic, IgG class, Fc gamma R-blocking antibodies were found in all of twenty-seven different batches of IV-IgG tested. The choice of lymphocyte donor was, however, found to be a critical factor in the detection of these antibodies. Lymphocyte donor panel studies indicated that Fc gamma R-blocking antibodies may in fact be autoantibodies. These auto-Fc gamma R-blocking antibodies may play a role in maintaining the normal immune response.

Po.68 **EXPERIMENTAL AUTOIMMUNE UVEITIS, (EAU) IN GUINEA PIGS: THE EFFECT OF CYCLOSPORIN A ON DTH RESPONSES TO RETINAL S ANTIGEN ON Ia ANTIGEN EXPRESSION BY INTRAOCULAR INFLAMMATORY CELLS**

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EAU closely resembles certain forms of human uveitis, in which autoimmunity to a soluble retinal antigen. "S" antigen is postulated. Dunkin-Hartley guinea pigs were immunised with highly purified bovine retinal S antigen (S Ag) in complete Freund's adjuvant and treated from day 0 either with cyclosporin A (CsA); 25 mg/kg per OS or vehicle. DTH skin tests carried out at 7 and 13 days showed maximal reactivity at 24 hr. CsA reduced the DTH response to S Ag and PPD and prevented vitreal inflammation. Lymph node and splenic lymphocytes from immunized guinea pigs showed a specific mitogenic response to S Ag which was profoundly suppressed by CsA. Responses to PHA, Con A and LPS were not so affected. Immunohistochemical staining with monoclonal antibodies to guinea pig 7 cells (CT6 and 7) and Ia antigen (CT 13.1), kindly provided by Dr. R. Scheper, Freie Universiuty, Amsterdam, showed that CsA inhibited the expression of Ia on choroidal inflammatory cells.

Po.69 **T & B CELL AUTOANTIGENIC EPITOPES ON MOUSE THYROGLOBULIN**

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We have used a panel of thyroglobulins (Tgs) from a variety of species to analyse and compare the epitopic specificities of monoclonal and polyclonal anti-Tg autoantibodies with the epitopes recognized by Tg-specific T cell lines and hybridomas. Monoclonal antibodies defined five specificities of which the dominant reactivity reflected the

polyclonal autoantibody profile, being largely specific for mouse Tg with a lower affinity cross-reaction with rat Tg. Tg-specific T cells defined three autoantigenic specificities, all of which were different to those recognised by autoantibodies. The molecular nature of these epitopes is currently being characterized.

Thus, in the mouse, Tg displays a minimum of five B cell epitopes and three T cell epitopes.

Po.70 **CYTOTOXICITY OF TUMOUR NECROSIS FACTOR FOR THYROID EPITHELIAL CELLS**

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The mechanisms by which end organ damage is effected in autoimmune disease remain incompletely defined. We have used FRTL-5, a TSH-dependent differentiated untransformed thyroid epithelial cell line, to study the cytotoxic actions of tumour necrosis factor (TNF) and other cytokines on thyroid cells. Recombinant human or murine TNF or TNF-containing sera (TNS) from rabbits or mice caused dose-dependent killing of FRTL-5. Pretreatment of FRTL-5 cells with gamma-interferon (gamma IFN) caused a rapid and dramatic enhancement of their sensitivity to killing by TNF. This increased sensitivity preceded the gamma IFN-induced expression of Class II MHC determinants on the epithelial cells by at least 12 hours. If these findings are representative of in vivo events, then such synergistic actions between cytotoxins and gamma IFN may mediate the initial epithelial lesion in autoimmune thyroid disease.

Po.71 **THE MECHANISM OF INHIBITION OF EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS (EAE) IN THE RAT BY MONOCLONAL ANTIBODY AGAINST CD4**

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Ongoing clinical signs of EAE in both rats and mice can be terminated by the administration, to diseased animals, of monoclonal antibody (MAb) which binds to the CD4 antigen present on helper/inducer T cells. How the MAb acts to inhibit disease is yet to be established. In data to be

presented, we provide evidence that anti-CD4 MAb prevents EAE in the Lewis rat by a mechanism which does not ablate or render anergic, the encephalitogenic $CD4^+$ cells or prevent the development of resistance to EAE, but which may inhibit the disease by preventing the function of already activated effector cells. Possible sites of action of the anti-CD4 MAb will be considered.

Po.72 **IMMUNOLOGICAL STUDIES ON THEILERS VIRUS INDUCED DEMYELINATION**

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Susceptible strains of mice infected with Theiler's virus have been used as a model for studying demyelinating disease. The relative contribution of the immune system to the demyelination process is unclear.

To investigate the role of T lymphocytes in the development of the disease, susceptible (CBA, SJL) and resistant (Balb/c) mice were selectivley depleted in vivo of T cell subsets (L3T4, Lyt2+ve cells), using specific monoclonal antibodies (Cobbold et al, 1984, Nature, 312, 548). The effect of this treatment on the course of the infection in the different strains of mice will be presented.

Po.73 **POLYCLONAL B CELL ACTIVATION IN BROWN NORWAY RATS AFTER EXPOSURE TO CADMIUM CHLORIDE**

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In the spleen of Brown Norway rats, injected with $CdCl_2$ (0.8 mg/kg; 5 days p. wk.), a transient increase was found in the number of IgM-plaque forming cells. A maximum was reached after 4 days (14 x background level of PFC's), followed by a sharp decrease to about 0.03 x background level at day 15. In comparison with control animals, increased ratios of W3/25+ve over Ox8+ve cells were found at day 4 and decreased ratios were found at day 15 of $CdCl_2$-treatment. Rechallenge with $CdCl_2$ after a 20 day period without cadmium injections could no longer evoke these reactions, indicating a suppressive mechanism induced during the primary treatment. No such responses were found in Lewis rats, despite a comparable accumulation of cadmium in liver, thymus and spleen. The responses to cadmium chloride seem very similar with the well-documented effects of mercuric chloride in BN rats. We are now searching for the presence of autoantibodies in cadmium-treated rats.

Po.74 PRE-TREATMENT WITH TYPE II COLLAGEN OR WITH 1 ALPHA 2 ALPHA 3 ALPHA COLLAGEN INFLUENCES SUBSEQUENT INDUCTION OF TYPE II COLLAGEN-INDUCED ARTHRITIS IN THE RAT

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Type II collagen, 1 alpha 2 alpha 3 alpha collagen and type IX collagen are all present in articular cartilage. An inflammatory arthritis was induced in Wistar rats by intradermal immunisation with native bovine type II or 1 alpha 2 alpha 3 alpha collagen emulsified in Freund's incomplete adjuvant but not by native type IX collagen. Serum antibodies to the respective collagens were found in the sera of immunised rats.

When either native type II collagen or native 1 alpha 2 alpha 3 alpha collagen (at the concentration used for intradermal immunisation) was administered intraperitoneally or intravenously three days before intradermal immunisation with native bovine type II collagen, the inflammatory polyarthritis was inhibited. The inhibition was observed as a delay in the onset of arthritis, a reduction in the severity of arthritis or a reduction in the number of arthritic rats. Prior administration of type I collagen (a non-articular-cartilage collagen) or buffer did not inhibit the arthritis.

Anti-native bovine type II collagen antibodies (G, A and M classes) were reduced by all the treatments that caused inhibition of the arthritis. The reduction in antibody response was not seen after pre-treatment with native type I collagen or buffer.

Antibodies raised to native bovine type II collagen or to native 1 alpha 2 alpha 3 alpha collagen cross-reacted with each other but not with type I collagen. Thus only pre-treatment of the rats with the immunising antigen or a cross-reacting antigen caused a reduction in humoral immunity to the immunising antigen and a reduction in arthritis. Results on pre-treatment of rats with type IX collagen will also be reported.

Po.75 ANTIBODIES FROM AUTIMMUNE CHRONIC ACTIVE HEPATITIS (CAH) & PRIMARY BILIARY CIRRHOSIS (PBC) RECOGNIZE DIFFERENT SPECIFICITIES ON LIVER MEMBRANE PROTEIN COMPLEX (LSP)

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Proteins from human LSP, rabbit LSP, and rabbit kidney equivalent (KSP), were separated by SDS-PAGE, transferred to nitrocellulose sheets and incubated with sera from 10 normal subjects, 6 CAH and 6 PBC cases. Normal sera reacted with several bands in human LSP, rabbit LSP and KSP, 4 CAH sera presented various specific bands with MW 22 to 70 KD. Specific bands with different MW were detected using rabbit LSP and KSP. PBC sera recognized two specific bands with MW 60 and 70 KD, only on human LSP. Thus anti LSP represent a population of antibodies with specificities for different components of LSP preparation.

Po.76 ANTIBODIES TO NUCLEAR PROTEINS IN SYSTEMIC LUPUS E. (SLE) & RHEUMATOID ARTHRITIS (RA)

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We investigated the specificity of circulating auto-antibodies in 11 SLE and 7 RA patients by means of ELISA on purified nuclear proteins related to DNA metabolism: DNA polymerase alpha, DNA dependent ATPase, DNA topoisomerase I, ssDBP, hnRNP, HMG, histones. SLE sera reacted with all antigens, especially with HMG ssDBP and with DNA enzymes. RA sera strongly reacted with DNA dependent ATPase, DNA polymerase alpha, ssDBP and hnRNP. The presence of autoantibodies to these nuclear protein may be related to impaired DNA replication and/or transcription and consequently to alterations of gene expression.

Po.77 **REACTIVITY OF HUMAN HYBRIDOMA LUPUS ANTICOAGULANT & ANTI-DNA ANTIBODIES WITH PLATELETS**

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Lupus anticoagulants (LA) have been associated with thrombocytopenia and thrombotic events. The present study assessed hybridoma LA and anti-DNA antibody reactivities with platelets. A solid phase radioimmunoassay was used to detect the direct binding of LA antibodies to platelets. Fifteen human hybridoma antibodies with either anti-DNA or LA activities were tested. All of the 8 antibodies with anti-DNA activity showed direct binding to platelets, while the 7 LA antibodies did not exhibit binding. The preincubation of DNA with platelets greatly increased the binding of the anti-DNA antibodies but did not affect LA binding. The addition of DNase to untreated platelets did not decrease the anti-DNA antibody binding but did abolish the enhanced binding due to exogenously added DNA. Preliminary studies performed using platelet aggregometry demonstrated that LA antibodies can cause platelets to undergo a shape change, while hybridoma anti-DNA antibodies had no effect. Phase contrast microscopy demonstrated that the 1 LA antibody studied caused advanced platelet morphological transformation. In summary, hybridoma LA antibodies may cause platelets to undergo morphological changes, whereas hybridoma anti-DNA antibodies appear to bind to platelet membranes without affecting platelet shape.

Po.78 **AUTOANTIBODIES TO A 56kd PROTEIN IN PATIENTS WITH MYOSITIS**

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Amongst the autoantibodies found in patients with SLE and myositis are those directed against nuclear components including a variety of ribonucleoprotein (RNP) complexes. Utilizing mammalian nuclear preparations enriched with RNP particles as the antigenic source for immunoblotting studies we have examined sera from i) 10 patients with myositis ii) 6 with SLE and myositis iii) 12 lupus patients with cerebral and/or renal disease iv) 8 SLE patients without myositis, renal or cerebral disease v) 8 patients with other myopathies. In 12 of the 16 patients with myositis

antibodies against a nuclear RNP protein of 56kd were found. In contrast only 2 of the patients in groups iii and iv and in none of the other controls were these antibodies detected. Antibodies to a nuclear RNP protein of 56 kd may be a useful marker for autoimmune muscle disease.

Po.79 **IN SITU CAPTURE OF SINGLE-STRANDED DNA-REACTIVE MONOCLONAL AUTOANTIBODIES PROMOTES RENAL FAILURE IN MRL MICE**

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Monoclonal autoantibodies derived from MRL mice failed, in general, to induce loss of kidney function when injected into normal mice. Such antibodies, however, administered to adult MRL/lpr mice accelerated the normal course of the disease leading to kidney dysfunction, weight loss, and in the most severe cases, death.

We conclude that: (I) Immune complexes (IC) trapped in kidney glomeruli can capture DNA-reactive antibodies of defined specificity circulating in the blood. (II) Both single and double stranded tracts of DNA are available on IC within the glomeruli. (III) Antibodies reactive with ss-DNA can cause kidney damage.

Po.80 **NEW METHOD FOR DETECTING ANTI LIVER CELL MEMBRANE ANTIBODY USING A HUMAN HEPATOMA CELL LINE**

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A simple technique for detection of serum autoantibodies that bind to liver membrane antigens on the surface of liver cells has been developed using Alexander cells (PLC/PRF/5). Alexander cells were incubated with test serum (1:160), washed, then adherent antibodies were detected by incubating with ^{125}I-Protein A. Specific binding to cell surface was calculated as the ratio between the counts with patients' serum divided by 2SD above the mean count obtained with serum from controls. The ratio was significantly higher in those with active autoimmune chronic active hepatitis (aCAH) (median 4.5, range 1.1-23.1) when compared to inactive cases (1.5, 0.3-4.1; $p<0.01$), to alpha-1-antitrypsin deficiency (1.2, 0.3-2.3; $p<0.01$) and to Wilson's disease (1.7, 0.3-2.9; $p<0.01$). The ratio in primary sclerosing

cholangitis was similar to active aCAH (4.2, 0.8-6.7), which would support recent evidence implicating autoimmune mechanisms in PSC. In 11 aCAH cases tested for anti-LSP (liver specific lipoprotein) antibody a positive correlation was found between Alexander cell binding assay and anti LSP titres ($r=0.56$, $p<0.02$). In 4 aCAH tested at diagnosis (4.8, 3-23.1) the ratio fell after effective immunosuppressive therapy (0.9, 0.6-4.5). Alexander cell binding assay provides a simple, rapid and sensitive technique to detect specific antibody to liver cell membrane which may help in the management of autoimmune liver disease.

IMMUNE REGULATION

Po.81 CELL MEDIATED IMMUNITY TO COLLAGEN TYPE I IN PERIDONTAL DISEASE; ROLE OF SUPPRESSOR CELL ACTIVITY

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In peridontal disease lymphocyte transformation with Type I Collagen correlates positively with disease score to a point of medium disease severity and then declines; this response is predominantly B cell in type. To study this further, peripheal blood cells from severely diseased patients were separated into mononuclear, T enriched and B enriched cell populations and one half of the mononuclear cells treated with anti Leu 2a and human complement (C). All the cell groups were then incubated with human Collagen Type I (3.125-200 µgm/ml) for 5 days at 37°C; lymphocyte transformation was assessed by uptake of ^{3}H thymidine. Responses in the mononuclear cells were elevated following anti Leu 2a + C treatment to levels approximating those observed in the B cell groups. It would appear that the depressed response to Collagen Type I in patients with severe peridontal disease is due, at least in part, to the activity of a suppressor cell subset.

Po.82 INTERLEUKIN 2 (IL 2) RESPONSIVITY BY PBMC OF PATIENTS WITH HODGKIN'S DISEASE, PREVIOUSLY TREATED & LONG-TIME OFF-THERAPY

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Thirteen patients with Hodgkin's disease (HD) previously treated, nine of whom long-time off-therapy, were studied for peripheral blood mononuclear cells (PMBC) response to IL 2 and for lymphocyte subpopulations by means of in

vitro cultures and monoclonal antibodies. The aim of the study was to ascertain the role played by the IL 2 in the impaired cell-mediated immunity of HD patients. PBMC cultures were performed with microtitre plate technique and ^{3}H thymidine uptake evaluation and the lymphocyte subpopulations analysis was carried out with optical flow cytometry. The results show a slight decrease of total T cells ($T3^+$), of the helper/inducer subset ($T4^+$) and of the $T4^+/T8^+$ ratio and a significant impairment of polyclonal responses to all the mitogens employed. As far as the IL 2 involvement in HD is concerned, our study suggests: 1) an impaired endogenous IL 2 production by T lymphocytes, 2) a most probable deficiency of the IL 2 receptor (Tac) expression and 3) a decrease in the number and/or of the function of NK cells, which are no longer responsive in vitro to IL 2. Our data seem to support the rationale for a therapeutic approach with IL 2 in controlled clinical trials also in HD patients, according to the in progress experiments in solid tumour patients.

This work was supported by the CNR (Italian National Research Council) Applied Project "Oncology" (Contr. No. 85.02133.44).

Po.83 IgA SPECIFIC T HELPER CELLS IN THE HUMAN COLONIC MUCOSA?

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The regulatory effects of mucosal T cells on production of IgA, IgG and IgM by colonic lamina propria cells (LPL) and peripheral blood lymphocytes (PBL) were investigated. Histologically normal colonic mucosal tissue from biopsy and resected specimens was used as the source of lamina propria lymphocytes (LPL). Resected mucosal cell populations were further separated into T (regulatory) and non-T (responder) cells by srbc-rosetting. Co-cultures at various ratios were established with or without pokeweed mitogen (PWM), and maintained for 6 days. The regulatory effects of biopsy isolates on PBL responder cells was also investigated. Ig production was estimated by ELISA of culture supernatants. In absence of mitogen, at all ratios tested (1:1 - 10:1), LPL T cells provided a dose-dependent helper effect for all Ig classes (up to 20-fold increase). With PBL responder cells, synergistic effects were observed for IgA (but not IgG or IgM) secretion with admixed LPL. Whereas PWM increased production of IgG and IgM with PBL responders, it had no stimulatory effect with either responder cell system

on IgA secretion: on the contrary it produced a slight suppression of response. The high levels of IgA produced and the inability of PWM to further stimulate IgA production suggests that the effects were due to IgA-specific T helper cells, which are already maximally stimulated in the normal colon.

Po.84 **IN VITRO IMMUNOGLOBULIN PRODUCTION BY ISOLATED HUMAN SMALL INTESTINAL MONONUCLEAR CELLS**

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Factors that control intestinal immunoglobulin secretion in the human small intestine are largely unknown. The regulatory role of T cells has been investigated by coculturing intestinal mononuclear cells (IMC), isolated from endoscopic duodenal biopsies, with autologous peripheral blood T lymphocytes (PBT). ELISA of culture supernatants showed IMC alone produced low levels of IgG; IgM and IgA mean values were 3.82 and 10.33 ($\mu g/10^6$ cells) respectively (n = 20). Peripheral blood T lymphocytes in the absence of mitogen provided helper effects for IgM and IgA production by autologous IMC at ratios of 10:1 (IgA $p<0.02$; IgM $p<0.05$). These results demonstrate that T lymphocytes exert an immunoregulatory influence on immunoglobulin production by human small intestinal lymphocytes.

Po.85 **EFFECT OF PREGNANCY PLASMA ON LYMPHOCYTE PROLIFERATIVE RESPONSES & GAMMA-INTERFERON BY AUTOLOGOUS LYMPHOCYTES**

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Previous studies had shown that when lymphocytes from pregnant women were cultured in autologous plasma their proliferative response was decreased compared to the post natal period. It was found that the antigen specific proliferation of post natal lymphocytes and gamma-interferon production by these lymphocytes was reduced in the presence of plasma taken during pregnancy compared to that harvested in the post natal period. Mixed experiments did not indicate that pregnancy plasma contained any suppressive factor. We concluded that the decreased responsiveness was due to a lack of the ability of the pregnancy plasma to support these in vitro lymphocyte responses.

Po.86 **ON THE REACTION TO AUTOLOGOUS LYMPHOBLASTS: EVIDENCE FOR ACTIVATION BY SECRETED FACTORS RATHER THAN INDUCTION BY AUTOANTIGENS**

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The ability to generate, if the presence of xenoantigens is excluded, a human Autologous Mixed Leukocyte Reaction has led to questions concerning its significance. Such issues cannot be resolved satisfactorily until the nature of the stimulation delivered to the T4+ responding cells is established. Since B lymphocytes, macrophages, monocytes and T and B lymphoblasts are all effective stimulators, surface HLA-D/DR has been proposed as the stimulating antigen. Using normal peripheral blood mononuclear cells, PHA lymphoblasts and PWM lymphoblasts to stimulate autologous responders, under completely autologous conditions designed additionally to eliminate carryover of mitogen, only PWM lymphoblasts were effective stimulators. This stimulation was independent of surface HLA-D, HLA-DR, IgG, and IgM, and evidence is presented that new or induced antigen expression is not responsible for stimulation; nevertheless, viable stimulator cells capable of protein synthesis are required. It appears that the PWM lymphoblasts release factors, neither IL-1 nor IL-2, that are capable of activating responder cells independently of the stimulator cells. The PWM lymphoblasts of roughly 50% of apparently normal individuals stimulate autologous responding cells, but for individuals with rheumatoid arthritis or atopic allergy the positive responders are more than 95%. It is suggested that the autologous response to PWM lymphoblasts and the release of activating factors is indicative of a hyper-reactivity in B cell activation, and may represent a predisposition to autoimmune phenomena.

Po.87 **MONOCYTE ACTIVITY & A POSSIBLE RELATIONSHIP TO BONE RESORPTION IN OSTEOPOROSIS**

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From experiments in vitro, it is clear that the calcium regulating hormones tend to affect osteoblasts more than osteoclasts. Nevertheless, in osteoporosis the balance of osteoclast and osteoblast activity is tipped towards the former, while no disturbances in calcium regulating hormones can be demonstrated reliably. At least in vitro, the major

regulators of osteoclasts appear to be of immune origin, these being monocytes, the prostaglandins that they secrete,

Po.89 LIPOSOMES AS IMMUNOLOGICAL ADJUVANTS IN VACCINES: COMPARATIVE STUDIES WITH ENTRAPPED [illegible]

Po.91 CHARACTERIZATION OF THE SPECIFIC T CELL FACTOR SMAF

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The immune response against tumour cells can be regulated by specific T cell factors. An example of one of these specific T cell factors is: Specific Macrophage Arming Factor (SMAF). It is produced under *in vitro* conditions when SL2 ($H\text{-}2^d$) immune C57BL ($H\text{-}2^b$) lymphocytes come in contact with these SL2 tumour cells. Gel-filtration and ion-exchange techniques were applied to purify SMA. SMAF has an approximate molecular weight of 60 kD and has an isoelectric point (pI) of 5.5. SMAF can be absorbed from the culture supernatant by plasma membrane of the SL2 tumour cells but not by plasma membranes of tumour cells with a different haplotype. The purification of SMAF has been difficult because the conventional purification procedures yield poor recovery of SMAF activity. Therefore we focused our attention on a T cell line that can produce relatively large amounts of SMAF in culture. In this paper we present the progress we have made on the purification of SMAF and the data SMAF producing T cell lines.

Po.92 ORAL TOLERANCE IN PIGLETS & PRERUMINANT CALVES

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Both piglets and calves can develop gastrointestinal disturbances which may be partly due to an allergic response to dietary protein. However, the reactions of piglets are generally transient, while those of calves are persistent and severe.

The ability of both species to control their immune response to dietary protein was studied by comparing reactions evoked by parenteral injection with soya protein in animals receiving either soya-containing or soya-free diets. Systemic soya-specific antibody responses were measured by ELISA, and cell-mediated reactivity by 24 hour cutaneous delayed hypersensitivity. No evidence was obtained for the existence of an oral tolerance mechanism in calves, but hyporesponsiveness to injection with soya was observed in soya fed piglets. We suggest that the development of oral tolerance in piglets and the apparent lack of this mechanism in calves may contribute towards the difference in severity of gastrointestinal hypersensitivity observed in the two species.

Po.93 CYCLOSPORIN A PRETREATMENT PREVENTS THE SUPPRESSION OF DELAYED-TYPE HYPERSENSITIVITY IN MICE IMMUNIZED WITH HIGH DOSE SHEEP ERYTHROCYTES

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When administered intraperitoneally to mice 2 days before immunization with a tolerogenic dose (10^9) of sheep red blood cells (SRBC), cyclosporin A strikingly augmented 4-day delayed-type hypersensitivity (DTH) footpad reactions. The stimulatory effect of CsA was observed over the dose range 5-200 mg/kg and was obtained in animals given the drug in one injection, up to 7 days before sensitisation. The augmentory effect of CsA was observed with the whole blood drug levels of 280-1260 ng/ml estimated by radioimmunoassay, at the time of immunization. Breaking of suppression was characterized by footpad swelling, intense mononuclear cell infiltration and increased deposition of ^{125}I-fibrinogen, following intravenous injection, within the challenge site. In addition, increased expression of procoagulant activity by spleen cells in response to antigen was observed. Cell transfer experiments showed that the CsA-enhanced DTH could be adoptively transferred to naive recipients. Additional transfers conducted at the time of antigen challenge, suggested that, under the conditions described, CsA inhibited the action of a population of suppressor cells normally effective during induction of DTH.

DENDRITIC CELLS & ANTIGEN PRESENTATION

Po.94 DENDRITIC CELLS IN THYROID AUTO-IMMUNE DISEASE

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Class II positive dendritic cells constitute a group of non-lymphoid mononucelar cells, which play an important role as antigen presenting cells in T-cell activation. Our objective was a study on the presence of dendritic cells in normal thyroid and thyroid affected auto-immune disease. For this purpose detailed immuno-histochemical studies were carried out with moabs L25, FD1, OKla, FK24 and S100. Our study presently includes 4 normal glands, 11 Graves' and 10 simple goiter. In all thyroid glands studied dendritic cells could be identified just underneath the follicular epithelium;numbers were larger in Graves' disease and simple

goiter. In normal thyroid glands dendritic cells were OKIa$^+$, FK24$^+$ and a very few S100$^+$. In simple goiter and Graves' disease dendritic cells additionally became L25$^+$ and FD1$^+$. Dendritic cells were not only present solitarily, but also in T-cell accumulations, which were found in 10/11 Graves' goiters and 2/10 simple goiters. These cells were FD1$^+$ and L25$^+$ as well, and were ultrastructurally similar to interdigitating cells from lymphoid organs.

In the marginal sinus of a thyroid draining lymphnode dendritic cells could also be demonstrated and they were positive for the thyroid auto-antigen thyroglobulin.

In conclusion our results suggest an important role for dendritic cells in thyroid auto-immune disease.

Po.95 INFLUENCE OF RETINOIC ACID ON DENDRITIC CELL FUNCTION

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The immunosuppressive drug Cyclosporine A inhibits the function of antigen-presenting dendritic cells (DC)[1]. Retinoic acid (RA) has immunomodulatory properties and we have studied its effect on the function of DCs in mixed leukocyte cultures. In vitro pulsing of murine splenic DR with RA blocked their capacity to stimulate allogeneic responses. Pulsing overnight with 10^{-12}M RA caused significant inhibition and a 2 hour pulse with 10^{-4}M RA completely blocked stimulation. RA treatment of responder lymph node cells reduced the background turnover and the response to allogeneic DC. Removal of DC from responder lymph node cells had a similar effect, and also reduced the effect of pulsing with RA. RA may therefore influence immune responses by modulating the function of antigen presenting cells.

1. Knight SC, Balfour B, O'Brien J, Buttifant L. Transplantation 41, 96-100

Po.96 DISTRIBUTION OF IMMUNE-ASSOCIATED (Ia) ANTIGENS ON HUMAN DENDRITIC CELLS

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Dendritic cells (DC) obtained from normal human peripheral blood were labelled with antibodies to HLA-DR, HLA-DQ and the antibody D1 μ (kindly provided by Dr. L. Poulter) which labels DC in tissue section. The labelled cells were then incubated with 20 nm colloidal gold particles coated with protein A, at either 4°C or 37°C, before examination by electron microscopy. Each antibody produced distinct labelling patterns with the anti HLA-DR and D1 μ antibodies particularly labelling the points of contact between DC and lymphocytes, or DG and macrophages. When gold labelled at 37°C the DR was internalised through deep 'channels'. However, there was only a small amount of superficial internalisation using the anti HLA-DQ and D1 μ antibodies. This technique has permitted the study of the distribution of antigens on individual DC and has demonstrated that substances bound to different Ia molecules may be processed differently.

Po.97 HUMAN BLOOD DENDRITIC CELL PREPARATIONS: EFFECTS OF GAMMA-INTERFERON ON HLA & MONOCYTE ANTIGENS

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Low density dendritic cell preparations obtained from normal human blood can present antigens. We show that these cells are all positive for class I (W6/32) and class II (HLA-DR, CA2) HLA antigens, but a proportion have monocyte markers (leu-M3, UCHMac1, 63D3). Two-colour flow cytometry showed that in vitro treatment with gamma-interferon (gamma-IFN, 200 IU/ml) reduces the level of monocyte antigens and increases the expression of class II on the same cells. However, gamma-IFN does not modify the already high expression of class II on the monocyte-negative cells.

Po.98 EFFECT OF CYCLOSPORIN A ON T LYMPHOCYTE & DENDRITIC CELL SUBPOPULATIONS IN PSORIASIS

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Sequential skin biopsies from 6 patients with severe psoriasis were studied during treatment with cyclosporin.

A subset of dendritic cells, HLA-DR+ but lacking the T6 antigen (DR+6-), was observed in lesional, but not uninvolved, psoriatic epidermis. They disappeared during treatment, before clinical improvement was apparent, and at a rate which correlated with clearance of psoriasis.

Total numbers of CD4 and CD8, anti HLA-DR+ CD8 T cells were significantly reduced in both epidermis and dermis prior to clinical improvement. In contrast, there was no decrease in epidermal HLA-DR+ CD4 cells, whereas these cells were reduced by an average of 68% in the dermis.

These findings further support the concept that T cells play a central role in the pathogenesis of psoriasis.

COMMITTEE ROOM 1

Po.99 ACCESSORY CELL REQUIREMENTS FOR PPD INDUCED LYMPHO-PROLIFERATIONS: A COMPARATIVE STUDY OF EPIDERMAL CELLS AND ADHERENT CELLS

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We have compared the ability of adherent peripheral blood mononuclear cells, PBMs (containing mainly monocytes) and epidermal cells (containing between 1 and 2% Langerhans cells), to support PPD induced proliferation of peripheral blood lymphocytes in humans.

Two lymphocyte populations were obtained from PBMs. One population routinely contained between 0.1 and 0.4% contaminating monocytes. The other contained no detectable monocytes. Lymphocytes were cultured in round-bottomed wells in the presence of PPD, with or without either epidermal cells or adherent cells.

Epidermal cells supported PPD induced lymphocyte proliferation only in the lymphocyte populations containing detectable monocytes. The ability of the adherent monolayers to support proliferation of both lymphocyte populations could be almost completely abolished after washing sufficiently with medium to remove weakly adherent cells.

The results suggest that in defined culture conditions more than one accessory cell is required to support PPD induced lymphoproliferation.

Po.100 HUMAN T CELL CLONES PRESENT ANTIGEN

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T cells cloned from the blood of a healthy donor by a well established protocol were screened for reactivity with the P20 epitope of influenza A HA1. As a control in antigen presentation experiments it was unexpectedly found that cloned T cells incorporated ^{3}H thymidine in response to synthetic peptides containing the P20 epitope, without the usual requirement of additional antigen presenting cells (APC). Such "autopresentation" was not observed when the antigen was in the form of whole influenza virus or haemagglutinin. Controls confirmed that no antigen or APC were carried over into the assay system. In a DRw53 restricted clone, autopresentation was severely inhibited by an anti DR antibody, with no effect on the clone's reponse to rIL2.

This is the first report of efficient antigen presentation by T cells to T cells.

INNATE IMMUNITY

Po.101 OPSONIC ACTIVITY OF PENTAGLOBIN

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Most intravenous preparations of immunoglobulins (Ig) are prepared from Cohn fraction II of human plasma and contain predominantly IgG. Antibodies elicited by Gram-negative pathogens, however, are frequently of IgM class. Opsonisation is one important defensive function of immunoglobulin.

We have used a chemiluminescence assay of neutrophil stimulation to assess the in vitro efficacy of Pentaglobin (Biotest; derived from Cohn fraction III and containing 76% IgG, 12% IgA and 12% IgM) to restore the opsonic activity for Gram-positive and -negative bacteria of sera from hypogammaglobulinaemic patients.

Pentaglobin opsonised all bacteria tested, particularly Gram-negative E.coli, Klebsiella and Pseudomonas ($p<0.001$).

Po.102 **TRANSFERRIN MEDIATED ANTIMICROBIAL ACTIVITY & OPSONISING CAPACITY OF PERITONEAL FLUID FROM PATIENTS UNDERGOING CONTINUOUS AMBULATORY PERITONEAL DIALYSIS(CAPD)**

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Patients undergoing continuous ambulatory peritoneal dialysis (CAPD) are prone to episodes of peritonitis caused principally by infection with Staphylococcus epidermidis. Since peritoneal macrophages from CAPD patients can usually ingest and kill opsonised S.epidermidis efficiently (submitted for publication), we have investigated whether the transferrin mediated antimicrobial activity and opsonising properties of the CAPD fluid are defective.

Addition of iron-free transferrin to CAPD fluid enhanced anti-bacterial activity to a degree that was inversely proportional to the original transferrin level but no such correlation was found with sera or laparoscopy fluid.

The ability of CAPD fluid to opsonise S.epidermidis for phagocytosis by peritoneal macrophages from CAPD patients was inferior to that of human serum, and there was a significant correlation between the levels of IgG and C3 in CAPD fluid with opsonic activity. No such correlation was found for laparoscopy fluid, which was superior to CAPD fluid in opsonising activity.

These results show that introduction of dialysis fluid into the peritoneal cavity of patients on CAPD dilutes potentially antimicrobial factors such as transferrin, IgG and C3 to levels at which they no longer function optimally and this may account for the susceptibility of CAPD patients to peritoneal infection.

Po.103 **IMPROVED SYSTEM FOR INVESTIGATING LEUKOCYTE LOCOMOTION IN VITRO**

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One of the major problems with many currently used assays of human polymorphonuclear leukocyte (PMN) locomotion in vitro has been that they rely heavily on visual counting techniques which are notoriously imprecise. Various modifications have been applied including radiolabelling the test cells, with associated disadvantages, or computer assisted image analysis which can prove expensive.

We have developed a system for investigating PMN migration through microporous filters using cheap multiwell disposable plastics. Within assay coefficients of variation are found to be in the range 5%-10% thus making it easy to exclude 'outlier' filters which have presented problems in other systems. The system is very simple and uses relatively few PMNs enabling large experiments to be conducted with the minimum of effort. Cells and filters may be recovered for further study after migration.

Po.104 **INHIBITORS OF NEUTROPHIL CHEMOTAXIS IN CIRRHOTIC ASCITES**

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Bacterial peritonitis remains a serious potential complication of cirrhotic ascites. We have studied the effect of ascites on neutrophil migration (Boyden method) in 17 cirrhotic patients. Ascitic fluid was poorly chemoattractant (33.6 ± 8.7 μm) for normal neutrophils compared to normal serum (95 ± 5.6 μm) ($p<0.001$) but was not significantly different from patients serum (35.6 ± 10.4 μm). Cell-directed inhibitors (CDI) were present in ascites from 4 patients, and chemotactic factor-directed inhibitors (CFI) were present in 4 patients. 2 patients had both CDI and CFI.

Inhibitors of neutrophil chemotaxis occur in cirrhotic ascites and may be relevant to the increased incidence of peritonitis in such patients.

Po.105 FIBRONECTIN & NEUTROPHIL CHEMOTAXIS IN LIVER DISEASE

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Plasma fibronectin levels were significantly lower than control in 74 patients with liver disease (245 ± 142 mg/L vs 353 ± 79 mg/L; $p<0.001$), and patients' serum was significantly less chemoattractant than control for normal neutrophils (45.9 ± 18.7 μm vs 76.3 ± 10.4; $p<0.001$). Migration distance correlated directly with plasma fibronectin ($p<0.01$) and inversely with disease severity ($p<0.001$). Addition of fibronectin to 10 sera with markedly subnormal levels normalised the values but failed to improve the subsequent chemotactic response.

Reduced circulating fibronectin does not appear to be the cause of the impaired serum chemoattractant activity observed in some patients with liver disease.

Po.106 NEUTROPHIL COMPLEMENT RECEPTOR 3 EXPRESSION IN PAROXYSMAL COLD HAEMOGLOBINURIA

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CR3 expression, plasma C3d levels and white cell count were monitored during cold induced haemolysis in a patient with paroxysmal cold haemoglobinuria (PCH). Free haemoglobin was detected in plasma 10 minutes after exposure to cold. This was associated with an 84% increase in CR3 expression on circulating neutrophils. Peak increase in CR3 expression was reached 30 minutes after cold exposure, and then fell gradually to pre-infusion levels. During this time, the white cell count fell by 58% from 4.3/nanolitre to 1.8/nanolitre. Plasma C3d levels increased by 53% over the same period. Neutrophil activation associated with CR3 expression may account for some of the symptoms encountered in PCH.

Po.107 LEUKOTRIENE B_4 GENERATION BY NORMAL HUMAN NEUTROPHILS FOLLOWING AN IgG-DEPENDENT STIMULUS

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When stimulated with the ionophore A23187 or with unopsonised zymosan, human neutrophils generate leukotriene B_4 (LTB_4), predominantly extra- or intra-cellularly respectively.

We now report that human neutrophils produced LTB_4, identified both intra- and extra-cellularly, when incubated with large non-phagocytosable IgG-coated particles (Sepharose 4B). The production of LTB_4 (measured by radioimmunoassay and validated by RP-HPLC) was dependent on both particle number and concentration of bound IgG. Release, maximal after 15-30 min co-incubation, was enhanced by prior neutrophil activation by f-met-leu-phe.

Comparable LTB_4 generation was stimulated by antigen (*Aspergillus fumigatus*) - coated beads sensitised with specific IgG-containing fractions obtained from sera of patients with allergic bronchopulmonary aspegillosis.

These results suggest a further mechanism by which neutrophils may be activated to produce potent inflammatory mediators.

Po.108 KINETICS OF ACTIVATION OF THE HUMAN MONOCYTE-LIKE CELL LINE, U937, BY LYMPHOCYTE CONDITIONED MEDIUM

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The kinetics of macrophage activation have been investigated by measuring the binding of homologous IgG to the human monocyte-like cell line, U937, cultured for up to 48 hours with unfractionated lymphocyte conditioned medium (LCM) prepared from mitogen stimulated human peripheral blood mononuclear cells.

Exponentially growing U937 cells cultured in the presence of 1% LCM showed an increase in monomer IgG binding Fc receptor density to (mean ± SD) 146 ± 32% of initial values after only 4 hours, compared with 95 ± 23% in control cells ($p<0.05$). Maximal enhancement of Fc receptor density was observed after 24 hours culture with 1% LCM (381 ± 85% of

initial values) and after 12 hours with 5% LCM (385 ± 111% of initial values). This suggests that this form of macrophage activation may occur more rapidly than previously assumed. Receptor density remained significantly elevated ($p<0.05$) in cells cultured with LCM for 48 hours.

Equilibrium association constants for receptor binding to monomeric IgG were significantly lower ($p<0.05$) in cells cultured with LCM for periods greater than 20 hours.

The kinetics of LCM induced activation of U937 cell binding of chemically cross linked oligomer of human IgG and of a triggered respiratory burst will be reported together with the effects of inhibitors of protein and mRNA synthesis on these forms of activation.

Po.109 **POLARIZATION AND RECEPTOR REDISTRIBUTION IN CHEMO-ATTRACTANT-STIMULATED HUMAN BLOOD MONOCYTES**

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Monocytes stimulated with chemotactic factors (FMLP, C5a etc.) change from a spherical to a polarized, locomotor, shape. Polarization is maximal 15-20 min. after stimulation and declines thereafter. It is accompanied by a ligand-independent redistribution of receptors to the cell head, e.g. Fc-rosettes form at the cell head in FMLP-polarized monocytes, but after 30 min. the number of rosetted cells declines, possibly due to loss of FcR by endocytosis or shedding. In contrast to earlier reports using pure attractants showing that only 60% of blood monocytes were stimulable, we found that mixtures of a pure factor (FMLP or C5a) with activated serum would induce motility in up to 90% of the monocyte population.

Po.110 **DIGESTION OF BACTERIAL MACROMOLECULES BY PHAGOCYTIC CELLS**

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The digestion of radiolabelled DNA, RNA and protein from ingested bacteria by human peripheral blood neutrophils and monocytes was compared. Although neutrophils digest very little DNA to small TCA-soluble molecules (<40 bases) compared to monocytes (1), they release up to 30% as large

M.W. fragments. However, neutrophils degraded all ingested RNA to small TCA-soluble molecules and were more efficient in this respect than monocytes. Bacterial protein was digested equally efficiently by both cell types.

Mepacrine hydrochloride inhibited both the ingestion of bacteria by monocytes and neutrophils and the ingestion of DNA to molecules <40 bases. Digestion of DNA to large M.W. fragments was not affected by the drug.

(1) Lamers MC, deGroot ER & Roos D (1981) Eur. J. Immunol. 11, 757

Po.111 **DEFECTIVE DEGRADATION OF BACTERIAL DNA BY PHAGOCYTES FROM PATIENTS WITH SYSTEMIC (SLE) AND DISCOID (DLE) LUPUS ERYTHEMATOSUS**

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Phagocytes from the peripheral blood of SLE and DLE patients are defective in digesting radiolabelled single-stranded DNA, but not RNA or protein, from ingested bacteria, when tested in an *in vitro* assay (see Po.110). Neutrophils from patients with SLE but not DLE digested less DNA than controls to large fragments (oligonucleotides > 40 bases). Monocytes from SLE and DLE patients digested as much DNA to large fragments as controls but released 15-35% less low M.W. DNA (<40 bases). These defects resulted in concommitantly larger amounts of high M.W. DNA being sequestered in patients' cells. Phagocytosis of bacteria was not defective in either disease.

Po.112 **EVIDENCE THAT THE REDUCED NUMBER OF NATURAL KILLER CELLS IN INSULIN DEPENDENT DIABETES IS GENETICALLY DETERMINED**

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Viruses may cause insulin dependent diabetes (IDD). We wondered if the number and function of natural killer (NK) cells, which are important in anti-viral defense, are disturbed in patients with IDD. We studied 15 recently diagnosed IDDs, 15 IDDs diagnosed more than 3 years previously, 17 non-insulin dependent diabetes (NIDDs) and 18

controls. We determined the number of NK cells (expressed as log. %) using Leu 11 monoclonal antibody and the function (in log. lytic units) concurrently using a 51-Cr release assay with K562 as target cells. We found that the number of NK cells was reduced ($p<0.005$) in recently diagnosed IDD (0.97 ± 0.05) as compared with controls (1.16 ± 0.05) and NIDDs (1.16 ± 0.07) but was similar to the level in longstanding IDD (1.05 ± 0.04). To establish whether the reduced NK cell number is genetically determined we studied 13 identical twin pairs discordant for IDD; we found that even the non-diabetic co-twins had a reduced NK cell number (0.93 ± 0.05). NK cell function was similar in all groups; while NK activity per cell was significantly increased in the recently diagnosed IDDs (1.63 ± 0.07) as compared with longstanding IDDs (1.26 ± 0.04) and controls (1.36 ± 0.07). In conclusion the reduced number of NK cells in IDD appears to be genetically determined while their activity at diagnosis is increased.

Po.113 **APPROACH TO THE DIFFERENCES BETWEEN NK, ADCC AND LDCC ACTIVITIES**

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There is a great interest in the role of NK cells in lymphoproliferative disorders. Frequently, an intense reduction of NK activity is seen in these neoplastic processes.

We have separated peripheral blood lymphocytes by discontinuous density gradients of Percoll. These fractions were obtained: a) very low density, b) low density and c) high density.

Each fraction was characterized according to morphological criteria by electron microscopy, phenotype with monoclonal antibodies OKT3, OKM1, Leu1, Leu11, LeuM1 and cytotoxic activities such as NK, ADCC and LDCC. Furthermore, in each fraction, T3- and Leu11- subpopulations were purified by cytotoxicity with complement, in order to analyze their functional activity.

We show that the ADCC activity can be mediated both by LGL of low density as well as T lymphocytes of low and high density. The NK activity is mediated only by LGL of low density and LDCC is mediated by T lymphocytes of high density.

A subpopulation of cells that express both monocytic markers and LGL morphology and/or T cell associated marker, may be implicated in ADCC activity. We are at the moment testing this hypothesis.

Po.114 **DECREASED MEMBRANE FLUIDITY AFFECTS SUSCEPTIBILITY OF TUMOUR CELLS TO LYSIS BY NATURAL KILLER CYTOTOXIC FACTOR**

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We recently demonstrated that natural killer (NK)-sensitive target cells are rendered resistant to NK-cell mediated lysis after rigidification of the target cell plasma membrane by lipid modulation. In the present study we show that lipid modulation of target cells does not affect the binding by these cells of NK cytotoxic factor(s) (NKCF) which is released from the NK effector cells upon recognition and binding of the target cells. The susceptibility of target cells with rigidified plasma membranes for killing by NKCF is, however, substantially reduced demonstrating that lipid modulation mainly affects post binding events in NKCR induced lysis.

Po.115 **IMPAIRED OXIDATIVE FUNCTION OF ALVEOLAR MACROPHAGES IN PATIENTS WITH LUNG CANCER**

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Alveolar macrophages (AM) were obtained, by bronchoalveolar lavage, from 14 patients with bronchial carcinoma and 17 control patients. They were purified by adherance and cultured overnight in medium 199 supplemented by 5% foetal calf serum (FCS) or 5% autologous serum (AS). H_2O_2 release following stimulation by phorbol myristate acetate was measured fluorimetrically. There were no differences in H_2O_2 release from AM of patients or controls when the cells were cultured in FSC. AS had no influence on H_2O_2 release from control AM but for AM from bronchial carcinoma patients AS resulted in 39% reduction in H_2O_2 release compared with FCS. We conclude that serum factor(s) impair AM oxidative functions in bronchial carcinoma patients.

Po.116 THE TRIGGERING OF AN OXIDATIVE BURST IN MACROPHAGES BY SOLUBLE IMMUNE COMPLEXES AND ANTIGEN-FREE OLIGOMERS OF SIMILAR SIZE

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Previous studies (reviewed, 1) have established that the efficiency of soluble immune complex uptake and ingestion by macrophages is enhanced by the capacity of the complexes to rearrange into larger aggregates at the cell surface, through the formation of additional antibody-antigen bridges. This aggregation renders complex attachment to the cell essentially irreversible and constitutes the determining factor in the clearance of complexes from an environment rich in competing monomeric IgG (2). In the present study, the contribution of aggregation at the phagocyte membrane to complex induction of an oxidative burst was investigated by comparing the lucigenin-enhanced chemiluminescence triggered by soluble immune complexes of guinea pig IgG2 anti-DNP antibodies with DNP_{18}-BSA, and by affinity cross-linked, antigen-free IgG2 antibody oligomers which were incapable of further aggregation. Using complexes and oligomers of the same average size under conditions which resulted in similar levels of binding, the complexes were found to be between two and four-fold more effective than the oligomers as inducers of an oxidative burst in guinea pig peritoneal macrophages. The implications of this finding will be discussed.

1. Leslie RGQ (1985) Immunology Today. 6, 183-187
2. Leslie RGQ (1985) Molecular Immunol. 22, 513-519

Po.117 ASSESSMENT OF IMMUNOLOGICAL ACTIVITY OF LUNG MACROPHAGES IN SARCOIDOSIS

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Early events in the development of sarcoid lung granulomata include a T helper cell infiltrate and an increase in the number of activated alveolar macrophages. We have measured an immunological function of these macrophages, namely the support of proliferation of T lymphocytes induced by the mitogen Con A.

Alveolar macrophages obtained from patients with active disease were found to have consistently greater ability to support T lymphocyte proliferation than those from patients with inactive disease. This proliferation response correlated significantly with the ratio of T helper to T suppressor cells in the lavage (r=0.73). In contrast, there was no difference between the capacity of blood monocytes from active and inactive patients to support proliferation. This heightened reactivity in alveolar macrophages in active sarcoidosis may contribute substantially to the development of the disease. This reactivity is not mirrored in the peripheral blood.

IMMUNITY TO INFECTION

Po.118 INVESTIGATION OF CULTURE FILTRATE ANTIGENS FROM ASPERGILLUS SPECIES BY ELISA TECHNIQUES

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Saturated ammonium sulphate pecipitates were prepared from filtrates of 3-week shake cultures of 10 pathogenic *Aspergillus* species grown in Czapek-Dox medium. Antisera raised in rabbits and CBA-mice against *A.fumigatus* and *A.niger* precipitates were each titrated against this series by ELISA to establish cross-reactivities.

Several cultures of *A.fumigatus* and *A.niger* were grown from single spores. Precipitates from each filtrate of a given species showed comparable cross-reactivity with heterologous rabbit antiserum.

ELISA plates coated with various lectins were used to capture specific carbohydrate ligands from these precipitates. Several *Aspergillus* species produce concanavalin A-binding components, reacting differently with the two rabbit antisera.

Po.119 **TRANSIENT ABROGATION OF IMMUNOSUPPRESSION IN A PATIENT WITH CHRONIC MUCOCUTANEOUS CANDIDIASIS (CMCC) FOLLOWING VACCINATION WITH CANDIDA ALBICANS**

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Defective T-cell function is considered central to the development of CMCC. Enhanced in vitro CMI responses follow intradermal innoculation with candida antigens. We therefore investigated the therapeutic potential of vaccination in a patient with CMCC.

Prior to vaccination the patient had reversed T4/T8 ratio, raised IgE and serum inhibitor(s) of lymphocyte transformation to candida antigens and mitogens. Vaccination induced temporary changes in these.

High levels of antibody to a 47KD antigen of C.albicans were demonstrated pre- and post-vaccination. This antibody is associated with recovery from systemic candidiasis. Its presence in our patient may explain why CMCC patients appear protected from systemic spread.

COMMITTEE ROOMS 3 & 4

Po.120 **PATHOGENICITY OF LISTERIA GENOSPECIES FOR GENETICALLY RESISTANT & SUSCEPTIBLE MICE**

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Mean lethal dose and bacterial growth kinetics of five genospecies of Listeria in C57BL/6 and Balb/c mice revealed that L.monocytogenes (sensu stricto) (L.m.) and L.ivanovii (L.iv.) were pathogenic in both mouse strains, while L.seeligeri (L.s.), L.welshimeri (L.w.) and L.innocua (L.in.) were avirulent. The C57BL/6 mice, as expected, were consistently more resistant to infection. Histological examination of livers from mice infected with L.m. and L.iv. showed large inflammatory and necrotic foci by the third day of infection and granulomatous lesions by 9 days p.i. Conversely, L.s., L.w. and L.in. caused only an early mild neutrophil infiltration which resolved within a

few days. Scanty Gram positive bacilli of all genospecies were seen in Kupffer cells early in infection. Additionally, clusters of bacteria were present in the inflammatory foci and occasional bacteria in some hepacytes after infection with L.m. and L.iv.

Po.121 **STRAIN-SPECIFIC MONOCLONAL ANTIBODIES TO CAMPYLOBACTER PYLORIDIS**

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Campylobacter pyloridis, isolated from homogenised gastric antral biopsies in 7 patients with chronic gastritis, were pooled and used as antigen for raising monoclonal antibodies. Hybridoma and subsequent clone screening was by ELISA using a C.pyloridis soluble antigen. Several IgM class antibodies, all specific for 4/7 of the isolated strains, have been produced. These antibodies do not cross react with C.jejuni or the campylobacter-like organism recently isolated from ferret gastric mucosa. The strain-specific antibodies produced should provide useful tools in helping to study C.pyloridis, and its suggested involvement in the pathogenesis of chronic gastritis.

Po.122 **CELL MEDIATED CYTOTOXICITY IN CATTLE IMMUNISED WITH LYMPHOBLASTOID CELL LINES PERSISTENTLY INFECTED WITH THE PROTOZOAN PARASITE THEILERIA ANNULATA**

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It is known that cattle can be protected against a lethal heterologous challenge of Theileria annulata by immunisation with Theileria infected lymphoblastoid cell lines. The mechanism of protective immunity induced by these cell lines is unknown, but current evidence suggests that it may be cell mediated.

We have investigated this possibility by immunising animals with MHC Class I defined bovine lymphoblastoid cell lines infected and transformed in vitro with a Moroccan strain of T.annulata. We examined the development and specificity of cytotoxic cells generated throughout primary immunisation and challenge. At day 9 after immunisation we observed a very specific cytotoxic response against the cellular antigens of the cell line used to immunise. After challenge the cytotoxicity detected was parasite specific and predominantly MHC restricted to the host.

Po.123 **HISTOPATHOLOGICAL EXAMINATION OF THE CELLULAR REACTIONS AROUND THE TRAPPED PARASITES OF SCHISTOSOMA MANSONI IN THE LUNGS OF IRRADIATED, IMMUNE & CONTROL RATS**

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Recent passive transfer experiments in rats have shown that irradiated (750 rad) and unirradiated recipients of immune serum manifest comparable levels of resistance to a challenge infection despite a greater than 85% reduction in peripheral blood leucocyte counts (Ford et al, submitted for publication).

Subsequently, histopathological examinations were undertaken to compare the inflammatory reactions around parasites in rat lung sections from both irradiated and normal recipients of immune and control serum. A variety of statistical data are presented concerning the number of inflammatory foci, numbers of free and trapped parasites present, and the size and cellular composition of foci in each of the four groups.

Po.124 **IMMUNITY TO SCHISTOSOMA MANSONI IN VIVO. II: DO POLYMORPHS, LYMPHOCYTES OR PLATELETS PLAY A VITAL EFFECTOR ROLE IN IMMUNE ATTRITION IN VACCINATED MICE?**

DAA Vignali, QD Bickle & MG Taylor

Dept Medical Helminthology, London Schl of Hygiene & Tropical Med, Winches Farm Field Station, St. Albans, Herts

Recent passive tansfer experiments in rats have shown that irradiated (750 rad) and unirradiated recipients of immune serum manifested comparable levels of resistance to a challenge infection despite a greater than 85% reduction in peripheral blood leucocyte counts. This suggests a primary role for radioresistant immune components such as macrophages and complement in the efferent arm of resistance to S.mansoni in rats (Ford et al, submitted for publication).

We have now also carried out experiments on the effect of irradiation on immunity in mice, this time studying the effect on immunity actively induced by infection with highly irradiated cercariae (20Krad). Five weeks after vaccination, mice were irradiated (650rad), and challenged three days later. It was found that irradiated mice developed levels of resistance comparable with unirradiated

controls, in spite of a marked reduction in circulating leucocytes (>90%) and platelets (>85%) during the skin and lung stages of parasite migration, and despite the complete abrogation of DTH response to S.mansoni antigens in vaccinated, irradiated mice as measured by foot-pad swelling. Histopathological examination of lung sections from these irradiated mice showed a marked reduction in the numbers of lymphocytes and polymorphs, particularly eosinophils, surrounding the parasites and in the lung tissue as a whole.

In conclusion, these experiments strongly suggest that neither polymorphs, lymphocytes nor platelets are involved in the effector arm of anti-schistosomiasis immunity in mice and rats.

IMMUNOCHEMISTRY

Po.125 **USE OF THE AVIDIN-BIOTIN SYSTEM TO IMPROVE IMMUNOLOGICAL METHODS WITH A SOLID-PHASE STEP**

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Adsorption of proteins to a solid-phase can reduce their immunological reactivity. Some anti-fluorescein (anti-FLU) monoclonal antibodies were found to lose their anti-FLU reactivity when absorbed to polystyrene. Indirect attachment of the antibodies by a protein-avidin-biotin capture (PABC) system resulted in enhanced retention of anti-FLU reactivity.

Covalent attachment to a matrix can result in loss of antibody activity. An affinity chromatography system was developed in which biotin-labelled antibody was reacted with the antigen-containing mixture. The antibody-bound antigen was then captured with avidin-agarose.

Research supported by CRSR-2-2455 (USDA)

Po.126 **DEVELOPMENT OF A LASER NEPHELOMETRIC ASSAY FOR THE MEASUREMENT OF C4d**

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The measurement of C4d allows the assessment of classical complement (C) pathway activation. Laser nephelometry is a semi automated, rapid and sensitive technique. The classical C pathway was activated in normal human serum (NS) with heat aggregated immunoglobulins, to generate C4d. The

C4d containing serum was then incubated with polyethylene glycol 6000 (PEG) at the final concentration of 12% for 60 mins at 4°C, to remove larger C4 related molecules. After centrifugation the supernatant contained only C4d, as shown by immunoelectrophoresis using an anti C4 intact antiserum. For laser nephelometry, standard and test samples were treated with PEG 12%, final concentration. A reference curve was produced by incubating double dilutions of the activated, PEG treated NS with 1:5 dilution of anti C4 intact antiserum. A value of 100 C4d U/ml was given to the activated NS as top standard. The optimum incubation conditions were found to be 2 hrs at 22°C, at a final PEG concentration of 2.4%.

Blood was collected in EDTA to prevent C activation in vitro and stored at -70°C. Twenty healthy volunteers (9 males, 11 females median age 31 yrs) and 50 patients with classical or definite rheumatoid arthritis (RA) (40 females, 10 males median age 48 yrs) were studied. The mean C4d value obtained in healthy subjects was 43.3 U/ml, while the mean value for C4d in the RA patients was 107.3 U/ml (Student's t test, t=5.3, p<0.001). A similar level of signficance was obtained when data were analysed with Wilcoxon's rank sum test. Twenty nine out of 50 (58%) RA patients had elevated C4d levels, being greater than 2SD above the mean of the control group. The levels of C3 and C4, the conventional measure of C, failed to show any activation.

The assay described can quantitate the C4d molecule present in biological fluids and provides a measure of the classical pathway activation. The asssay can discriminate between normal subjects and patients with ongoing activation of the classical complement pathway.

Po.127 **PROSTAGLANDINS MAY CAUSE INHIBITION OF ANTIBODY AFFINITY MATURATION IN MICE**

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Spleen cells from mice, genetically selected to produce either high or low affinity antibody responses, were stimulated with various mitogens and the kinetics of the responses analysed. Results suggested that prostaglandins may play an important role in the control of the cellular interactions in these mice.

In order to test this hypothesis, a course of indomethacin (a prostaglandin inhibitor) was given while assessing affinity maturation of antibody responss to an antigen injected in Freund's complete adjuvant. It was found that the affinity of anti-HSA antibdoy from low affinity NM mice (that are usually unable to show affinity maturation to antigen injected in adjuvant) matured under the influence of indomethacin. This finding was reflected by a difference in the in vitro PGE2 production by adherent spleen cells from the different lines of mice.

Po.128 **ASSESSMENT OF THE AFFINITY OF ANTIBODIES TO TETANUS TOXOID BY A MODIFIED IgG SUBCLASS-SPECIFIC ELISA**

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We have recently reported[1] that low affinity antibody responses to tetanus toxoid in man are associated with an antibody response that is predominantly IgG4 whereas high affinity responses are associated with the IgG1 subclass. This may be due to affinity differences between the IgG subclassses, suggesting that IgG4 antibodies are intrinsically of low affinity. However, another possible explanation is that IgG4 antibodies are produced when low affinity antibody responses occur in any IgG subclass, perhaps indicating active T cell suppression. In order to try and answer this question, we have used a modified, subclass-specific ELISA, performed in the presence and absence of diethylamine, to measure the affinity of IgG1 and IgG4 antibodies to tetanus toxoid, without having to purify the different antibody populations.

1. Devey ME et al (1985). Immunology, 55. 565

Po.129 **USE OF THE AFFINITY DEPENDENCY OF THE ELISA FOR DETERMINING RELATIVE ANTIBODY AFFINITIES**

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It can be predicted from the Mass Law that the affinity (Ka) dependency of an assay for specific antibody depends on antigen concentration. Studies with anti-fluorescein monoclonal antibodies demonstate that estimation of antibody by the ELISA depends on the antibody affinity and the amount of antigen on the microtiter well. These data were used to

develop an ELISA-based method for estimating the relative functional Ka (rfKa) of antibdoy samples. The rfKa values obtained for six IgG_1 monoclonal antibodies correlated with Ka estimates obtained by dissociation rate analysis.

Research supported by HL22676 (NIH) and CRSR-2-2172 (USDA).

Po.130 **MONOCLONAL ANTIBODIES TO TETANUS TOXOID & TO THE PURIFIED CHAINS**

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Unlike most bacterial toxins, the action of tetanus toxin at a molecular level remains essentially unknown. Structurally, the toxic has a molecular weight of 150,000 and comprises two polypeptide chains, heavy and light, joined by disulphide bonds. The chemistry of the two chains is being investigated to verify similarities in their amino acid sequence[1].

Monoclonal antibodies directed agains whole toxin, toxoid and the purified chains have been raised to study common epitopes on both chains, the galglioside-binding site and the nature of the interaction between the two chains in the intact toxin molecule. A versatile, competition assay using biotinylated antigens and streptavidin conjugated horseradish peroxidase has been developed to detect those antibodies which recognise different determinants on the same antigen and to study the specificity of the antibodies for a particuar sequence.

1. Taylor CF, Britton P and van Heyingen S (1983) Biochem. J. 209, 897-899

Po.131 **MONOCLONAL ANTIBODIES TO SYNTHETIC PEPTIDES REPRESENTATIVE OF RAT IgE SEQUENCES REACT WITH NATIVE AND HEAT TREATED IgE**

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Previous work in this laboratory demonstrated that polyclonal rabbit antisera directed against seven synthetic peptides representative of sequences within the CH3 and CH4 domains of rat IgE react specifically with native and heat treated rat IgE. (1).

Monoclonal antibodies (Mab) have now been raised to some of these sequences and have been used to investigate the heat sensitive cytophilic site found in five rat IgE immunocytomas. The ability of the Mabs to block binding of rat IgE to mast cells *in vitro* has also been studied. These results will be discussed in relation to those obtained from similar experiments using polyclonal antisera.

(1) DR Stanworth, DS Burt, GZ Hastings (1986) Synthetic peptides as antigens. Wiley, Chichester (Ciba Foundation Symposium 119) p226-244

Po.132 **AN ELISA FOR DETECTION & CHARACTERISATION OF ANTIBODIES TO DISULPHIDE-CONJUGATED D-PENICILLAMINE**

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Drug-protein conjugates incorporating D-penicillamine (PA) disulphide-linked to HSA and KLH were synthesised by a range of oxidative procedures, and optimum conditions for conjugation were defined. Synthetic conjugates were employed as coating antigens in an ELISA for anti-PA antibody. IgG anti-PA antibody was detected by ELISA in the sera of 2/3 rabbits immunised by injection of PA-S-S-KLH in Freund's Complete Adjuvant. Binding of IgG to the antigen (PA-S-S-HSA) was inhibited by unconjugated PA, PA-disulphide, and PA-cysteine. Inhibition was also produced by protein conjugates incorporating PA but not by the same unconjugated protein. No inhibition of binding was produced by PA-acetone adduct nor by the structurally related sulphydryl drug captopril, thus demonstrating specificity of the antibody for the free sulphydryl and disulphide-conjugated forms of PA. IgG, IgE and IgM anti-PA activity could not be detected by the same method in sera from 32 rheumatoid patients receiving the drug.

Po.133 **HLA-D REGION ALPHA CHAIN mAbs: SEROLOGICAL CROSS-REACTION BETWEEN AN ANTI-DP ALPHA CHAIN mAb & SMOOTH MUSCLE PROTEIN**

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Two mAbs 3C3 and 1B5 which respectively define HLA-DP and DR alpha chain subunits were immunocytochemically characterised on fixed paraffin embedded human tissue

sections. Slight differences in reactivity were observed on subpopulations of lymphoreticular cells (expected to express class II antigens) as well as on different epithelia. mAb 3C3 showed additional strong staining of normal smooth muscle which from immunoblot studies was shown to be against a smooth muscle actin binding protein, filamin, which is structurally and functionally unrelated to class II molecules. These studies emphasise the possibility that mAbs may recognise protein products distinct from their main or expected targets and also demonstrate the value of tissue section analysis for screening mAbs.

Po.134 **BINDING OF IMMUNOGLOBULIN Fc TO CATIONIC PROTEINS**

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The interaction of cationic (basic) proteins with IgG, IgA and IgM was investigated by solid phase radioimmunoassay. All these immunoglobulins showed avid binding, IgM giving the strongest reaction, followed by IgA and then IgG. Fc fragments of IgG gave binding, but $F(ab')_2$ fragments from the three main Ig classes did not, showing that the Fc region is the active part of the molecule. The effects of changes of ionic strength and pH are compatible with the interaction being ionic, and are similar to those seen between immunoglobulins and both C1q and cationic ion exchange gels. The addition other serum proteins resulted in marked inhibition of the interaction.

In addition to RIA, the interaction could be shown by Ouchterlony gel precipitation, aggregated IgG giving strong lines with histone and poly L lysine.

The significance of these results for interactions of immunoglobulins *in vivo* and *in vitro* will be discussed. The possibility of two site antigenic recognition and an analogy with the T cell antigen receptor will also be described.

Po.135 **SPLENIC CLEARANCE OF ANTIBODY COATED ERYTHROCYTES IN DECOMPLEMENTED RATS. INFLUENCE OF RED BLOOD CELL ANTIGEN NUMBER ON THE INHIBITORY EFFECTS OF IMMUNE COMPLEXES ON Fc DEPENDENT CLEARANCE**

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The splenic component of the mononuclear phagocyte system was investigated in decomplemented rats by determining the clearance from the blood of erythrocytes coated with a monoclonal antibody (R3/13). The infusion of soluble immune complexes (IC's) at an appropriate time during the erythrocyte clearance produced a significant increase in the $T\frac{1}{2}$ of the antibody coated cells. Immune complexes formed with the $F(ab')_2$ fragment of the rabbit antibody did not have any significant effect.

The influence of red cell antigen number on the behaviour of erythrocytes sensitized with R3/13 was studied by comparing the clearance of DA and (DA x PVG) F1 erythrocytes. F1 erythrocytes, with only half the number of specific antigens on their surface that bind R3/13 antibody were cleared more slowly by the spleen than the DA erythrocytes. Both cell suspensions were equally susceptible to inhibition by soluble IC's.

These studies show that the number of specific antigens on the red cell surface influences the rate at which sensitized cells are removed by splenic macrophage Fc receptors but not their susceptibility to inhibition by IC's.

Po.136 **FIBRONECTIN IS A CONSTITUENT OF IMMUNE COMPLEXES IN RHEUMATOID DISEASES**

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The glycoprotein fibronectin, a non-specific opsonin, binds to C1q, hyaluronate and cells. We have investigated whether fibronectin is integrated into immune complexes *in vivo*.

Evidence from gel filtration, 2D-immunoelectrophoresis and SDS-PAGE demonstrated fibronectin was a constituent of circulating immune complexes in rheumatic diseases and was present in low molecular weight forms. Fibronectin was also

a constituent of immune complexes formed in vitro. In polyethylene glycol-treated rheumatoid arthritis serum and synovial fluid the amount of precipitated fibronectin correlated with the amount of precipitated immunoglobulin.

The association with fibronectin may be important for immune complex function.

MOLECULAR GENETICS

Po.137 ANTIBODIES TO T CELL ANTIGEN RECEPTOR BETA CHAIN FAMILIES DETECT MONOCLONAL T CELL PROLIFERATION

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The T cell antigen receptor is constructed from independent gene segments much like those used to assemble immunoglobulin genes. One of the receptor's two protein subunits, the beta chain, uses a limited number of variable region segments. The product of these V region segments can be identified by monoclonal antibodies and can be used to define populations of normal T cells which use the same V beta gene segments. Here we show that these antibodies can be used to define monoclonality or its absence in T cell populations. The malignant nature of T cell proliferations can be directly diagnosed in tissue sections and intact cell suspensions. It should also make it possible to quantitate changes in malignant populations in response to therapy.

Po.138 THE CHROMOSOMAL LOCATION OF THE HUMAN CD2 GENE

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The chromosomal location of CD2 has been defined using somatic cell hybrids and in situ hybridisation. Southern blotting analysis of human genomic DNA cut with one of the restriction endonucleases, EcoRI, Hind III, PstI or BamHI and probed with a full length 32-P-labelled CD2 cDNA revealed more than one band in each case. BamHI which produced three bands of 9, 7, 4 and 4.2kb was used for chromosome mapping with somatic cell hybrids. The CD2 probe produced a signal with a high degree of concordance for human chromosome 1.

In particular, the hybrid F4sc13c14 which contains the short arm only of the human chromosome 1 was positive. The location of the CD2 gene to 1p was confirmed by in situ hybridisation. Thus, changes in regulation and/or expression of the CD2 gene would be expected to involve alterations of the short arm of chromosome 1.

ADDITIONAL CONTRIBUTIONS

Po.139 CLASS II MHC ANTIGEN EXPRESSION ON UVB IRRADIATED HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS

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The effects of UVB irradiation on human class II MHC antigen (HLA-DR, HLA-DQ, and HLA-DP) expression by lectin-stimulated peripheral blood mononuclear cells have been examined in vitro. Cells exposed to 8-16 Joules/m^2 of UVB showed a UV dose related increase in expression of all three class II antigens following 60 hrs in culture. Maximum HLA-DR expression was achieved with a lower UVB exposure than HLA-DQ and HLA-DP expression. Exposure exceeding 25 Joules/m^2 resulted in decreased expression of all three class II antigens. The results indicate the UVB-induced modulation of class II antigen expression may provide a useful tool for examining the role of these molecules in immune cell interactions.

Po.140 ANTIGENICITY & IMMUNOGENICITY OF SYNTHETIC RENIN PEPTIDES: TOWARDS CONSTRUCTION OF A TOTALLY SYNTHETIC RENIN VACCINE

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Renin is an aspartyl protease which catalyses the first and rate-limiting step in the conversion of angiotensinogen into the pressor octapeptide angiotensin II. Specific blockade of the renin-angiotensin system can be obtained by both synthetic inhibitors and by passive or active immunization against renin. In this report we present preliminary data directed towards the development of a totally synthetic anti-renin vaccine. Seven peptides corresponding to predicted potential immunological epitopes of renin were synthesized. These peptides were covalently conjugated or copolymerized directly to the synthetic

adjuvant muramyl dipeptide (MDP) or to it analog MDPLys. All of these conjugates were characterized by HPLC. Their antigenicity was studied by using polyclonal and monoclonal anti-renin antibodies. The immunogenicity of each conjugate was tested in vivo and anti-peptide or anti-renin antibody titers were determined by ELISA together with their isotypic pattern.

Results demonstrate that a) the antigenicity of the peptides is enhanced after coupling to MDP, b) nevertheless the immunogenicity is affected by the number and location of MDP molecules coupled per molecule of peptide.

Po.141 **REVERSAL OF NEGATIVE SIGNALLING IN IMMATURE B LYMPHOMAS BY T CELL FACTORS**

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B lymphomas have proven to be valuable models for both positive and negative signalling via surface immunoglobulin (Ig) receptors. One of us recently reported on the inhibition of growth of three independently derived murine B cell lymphomas by monoclonal or polyclonal anti-Ig reagents. In contrast, the growth of these cells is unaffected by antibodies directed at either MHC class I or II markers expressed on these lines. We now describe the ability of T cell derived lymphokines to modulate the anti-Ig mediated growth inhibition. Thus, cloned alloreactive or OVA-specific T cell lines produce a factor or factors which enable these immune B lymphomas to maintain in vitro growth in the presence of normally inhibitory concentrations of anti-Ig reagents. The nature of these T cell factors is under investigation.

Po.142 **FUNCTIONAL HETEROGENEITY OF HLA-CLASS II DETERMINANTS**

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The genetic basis of lymphocyte activation in relation to the generation of cytotoxic/suppressor cells was investigated. Experiments were performed to define the relevant HLA Class II subregion determinants responsible. The following results were obtained: (1) In investigating the basis of HLA-Dw assignments it was found that responder cells do not have to share HLA-DQ antigens with stimulator

homozygous typing cells to give a typing response. (2) The common HLA-DR/DQ associations observed in the HTC's correspond to different patterns of linkage disequilibrium in different populations. (3) The generation of cytotoxic effectors and suppressor functions were blocked by anti-DQ and not anti-DR monoclonal antibodies. (4) The correlation between restriction fragment length polymorphisms obtained with DQ probes did not correspond to HLA-Dw assignments. Conclusion: the role of HLA-DQ as compared with HLA-DR subregion determinants appears to be one of the modulating the total T cell response by controlling proliferation of suppressor and cytotoxic cells and does not contribute significantly to HLA-Dw assignments.

Po.143 **INFLUENCE OF HLA MATCHING IN CARDIAC ALLOGRAFT RECIPIENTS RECEIVING CYCLOSPORIN A & IMURAN**

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The records of 204 patients who received Cyclosporin A and Imuran following cardiac transplantation, were analysed. Their ages varied from 6 to 63 years (mean = 52). There were 171 males and 33 females. ABO blood grouping was the only criteria used for donor/recipient selection. No attempt was made to match for HLA antigens. The overall graft survival was 76% at 1 year and 74% at 2 years. Previous transfusion (cardiac operation), age of recipient or original cardiac disease did not significantly influence graft survival. The effect of matching for Class I (HLA-A,B), Class II (DR, the second DR subregion series including DRw52 and DRw53 (SecDR) and DQ) on graft survival were determined. Matching for Class II antigens appeared to have a marked influence on the two year graft survival (84% for 1 DR mismatch and 68% for 2 DR mismatched p=0.05). Similar influence of HLA-DRw52/53 matching on a two year survival was observed (76% for 0 mismatch and 68% for 1 mismatch). Matching for Class I antigens alone did not significantly influence the outcome. However, when the combination of Class I (HLA-B) and Class (HLA-DR) were analysed, there was a significant influence on the two year graft survival suggesting a synergistic effect of Class I and Class II matching. It is concluded that HLA matching can have a profound influence on the medium term results of cardiac transplantation.