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Abstracts of Papers Presented

STUDIES ON A NEW MOUSE MACROPHAGE MEMBRANE MARKER DEFINED BY THE ANTIBODY MUM-4.
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A new monoclonal rat antibody, MUM-4, reacts with a membrane antigen on peritoneal mouse macrophages. MUM-4 is strongly expressed on virtually all resident peritoneal macrophages but is hardly detectable on peritoneal macrophages four days after an intraperitoneal injection of thioglycollate or killed Propionibacterium acnes organisms. The antigen is not detectable neither on blood lymphocytes nor on monocytes or spleen dendritic cells. The distribution of MUM-4 on tissue macrophages is in the process of being elucidated, but preliminary results indicate that only few tissue macrophages carry detectable amounts of MUM-4 antigen on their surface. The staining pattern observed with the MUM-4 antibody is unlike any other known to us, and the antibody thus seems to represent a new specificity. The MUM-4 epitope is present on resident peritoneal macrophages of all mouse strains studied. Characterizing the MUM-4 antigen by immunoprecipitation and Western blotting has proved difficult, which probably reflects structural features of the molecule. Recent results regarding the biochemical characteristics of the antigen will be presented.

Immunoglobulins as modulators of protein reactivities
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We have examined the modulating effects of human immunoglobulin on the reactions of specific antibodies with their antigen. The effects of non-immune polyclonal human IgG and/or rheumatoid factors from rheumatoid sera were estimated immunologically in two conventional antigen-antibody systems with polyclonal antibodies (HSA-rabbit anti-HSA and tetanus toxoid (TT)-human anti-TT). Using enzymes (glucose-6-phosphate dehydrogenase and human placental alkaline phosphatase) as antigens for polyclonal rabbit antibodies, effects on biological activities could be followed. Non-specific immunoglobulins were found to inhibit the binding of antigen by specific antibodies of both human and rabbit origin. Furthermore, human immunoglobulins were also able to modify the composition of preformed antigen-antibody complexes. The observed changes could not be ascribed only to the activities of specific antibodies in the immunoglobulin preparations.

In addition, we have also observed some odd interactions of immunoglobulins with affinity ligands which further indicate that immunoglobulins may interact with each other and with other proteins not only as antibodies against antigens, but also through interactions which are distinct from antigen-binding. A network of such "non-immunological" interactions would be of great importance in providing suitable conditions for physiological protein activities. It is also easy to conceive a regulatory function of immunoglobulins similar to the allosteric regulation of, for instance, enzymatic activities through this kind of interactions.
DIRECT ACTIVATION OF B CELLS BY MOUSE MAMMARY TUMOR VIRUS

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The primary target of infection with Mouse Mammary Tumor Virus (MMTV) is the B cell. Recently it has been shown that successful infection is dependent on the expansion of B cells carrying integrated MMTV by an immune response involving T cells reactive with the superantigen expressed by MMTV. Since retroviral integration is dependent on DNA synthesis this means that either MMTV infects the subset of B cells being in cycle at the time of infection or that MMTV can directly activate B cells in an superantigen-independent manner. We have analysed early events after local foot pad injection with infectious MMTV(SW). Already within the first 18 h after injection there is a pronounced polyclonal activation of B cells in the draining popliteal lymph node as shown by the increased expression of the early activation antigens CD 69 and B7-2 which is independent of the presence of T cells. This suggests that MMTV can directly activate B cells, most likely by binding to its cellular receptor on the B-cell. Thus, binding of MMTV to its receptor may serve both as a means of entry into the cell and as an activation event for the following integration.

Detection of proliferation and cytokine production in whole-blood cultures in vitro after stimulation with measles antigen.

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Measles remains to be one of the most important child health problem in developing countries. Studies of protective immune responses and identification of relevant B- and T-cell epitopes are required for a better understanding of immunity to measles. We have established techniques to measure antigen specific T-cell responses in whole-blood cultures from capillary specimens. These techniques were used to study proliferation and TH1/TH2 heterogeneity in response to various measles antigens when stimulating lymphocytes from immune adult donors or from children before and after vaccination. Separated lymphocytes from adult immune donors proliferated in response to crude measles antigen, i.e. Complement Fixation Agent (CFA), Andersson, C. Ardalvin, and H. Acha-Orbea. However such response was not detected when stimulating whole-blood cell cultures obtained from vaccinated children. T-cell activation can be manifested by cytokine secretion rather than proliferation, we therefore measured IFN-γ in supernatants from cell-cultures stimulated with CFA. Increased, dose dependent, IFN-γ production was detected by ELISA in cultures with separated lymphocytes from adult immune donors, as compared to control antigen and medium alone. Whole-blood cultures from vaccinated children are presently being examined in the same way. We are currently refining the technique for detection of low amounts of IL-4.

Expression of functional vascular adhesion protein-1 (VAP-1) is upregulated in inflammatory skin diseases in man

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The adhesion of leukocytes to vascular endothelium and their migration into tissues is mediated by adhesion molecules on the endothelial cells and leukocytes. Vascular adhesion protein-1 (VAP-1) is a 180/90 kDa endothelial molecule expressed most prominently in high endothelial cells in peripheral lymph node (PLN) type lymphatic tissues. The expression of VAP-1 is upregulated in inflammation at other sites as well. VAP-1 mediates lymphocyte binding to peripheral lymph nodes, tonsil and synovium. In the present work we examined the expression, structure and function of VAP-1 in normal and inflamed skin. In psoriasis, lichen ruber planus, pemphigoid and allergic lesions, VAP-1 was significantly upregulated. The expression of VAP-1 was also clearly increased in the macroscopically healthy skin of the skin disease patients. The VAP-1 molecule induced in skin diseases is identical in size to that found in tonsil HEV and is also modified with abundant sialic acid residues. VAP-1 in inflamed skin is functional since inhibition with an anti-VAP-1 mAb IB2 causes a 70% reduction in lymphocyte adhesion to vascular endothelium. In conclusion, VAP-1 is likely to contribute to lymphocyte homing to inflamed skin.

Fetal Antigen 1 (FA1) a new member of the EGF Superfamily, and a potential autoantigen in IDDM (type 1 diabetes)

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Fetal Antigen 1 (FA1) was first described by Fay et al., 1988 Eur.J.Obstet.Gynecol.Repro.Biol. 29, 73-85, and has recently been characterized with respect to primary structure and glycosylation by Jensen et al., 1994 Eur.J.Biol.Chem. 225, 85-92. In the context of diabetes there are striking similarities between human FA1 and the 38 kDa insulin secretory granule protein isolated from the rat (Roep et al., 1990 Nature 345, 632-634) with respect to Mr, and the tissue expression. The 38 kDa has been described as a major autoantigen in IDDM. We have established a murine animal model for the study of the biological significans of FA1 including: (i) purification of mFA1, (ii) production of antibodies, (iii) immunohistochemical location studies, (iv) establishment of ELISA technique.

In brief the preliminary data are:

• murine amniotic fluid contains 2500 times more mFA1 than normal mouse serum.
• fetal serum day (16-21) contains mFA1 at about the same level as amniotic fluid.
• the content of mFA1 in sera from pregnant mice increases at least 30 times before delivery and decreases in less than 6 hours after delivery to normal range of mFA1 in serum at adult mice.
• newborn mice have a mFA1 serum level 2500 times that of for adult mice decreasing to normal range at sexual maturity.
T-cell Recognition of Mouse Type-II-Collagen in Mice

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[partial reference]

Recognition of one or more epitopes on the mouse type-II-collagen (CII) by autoreactive T-cells is decisive in developing collagen-induced arthritis (CIA) in mice. The main aim of this project is localization of T-cell epitopes on the mouse CII, a matter of importance not only for understanding the nature of CII autoimmunity in the mouse, but also of importance in connection with clinical trials.

We have two tools for the epitope analysis: starting from mouse type-II-collagen cDNA, kindly given us by Professor Vuorio (Turku), we cloned and expressed segments of the mouse CII gene. Some 80% of the mouse CII protein sequence is covered by engineered peptides expressed as fusion proteins with the maltose-binding protein. These allow one to identify cryptic epitopes, i.e. epitopes not revealed by immunization with intact protein. In addition we obtained a series of synthetic peptides, covering the same 80% on the protein, for epitope mapping.

We localized I-Aq restricted autologous epitopes on the mouse type-II-collagen which have not been earlier identified. One epitope, giving the highest response, has been characterized further: T-cell(s) responding to it appear to be endogenously primed and upregulated during inflammation, suggesting a role for this epitope in the pathogenesis of CIA. Furthermore, epitopes identified in this study share a common binding motive to the I-Aq molecule.

The capacity of adjuvants to stimulate antigen-presenting cells (APC) to produce cytokines is likely to be important during the initiation of the immune response. Iscoms and its matrix have been reported to induce the production of cell-bound and secreted IL-1. Purified components of Quillaja extracts have been used to develop novel adjuvant formulations based on iscoms. We studied the capacity of four new matrix formulations to stimulate production of IL-1 in an in vitro system. Murine peritoneal cells were cultured with various concentrations of the matrixes and the supernatants were tested for IL-1 by immunoassay. The most efficient formulation was 7.0.3. The influence of the physical conformation of the adjuvant on the capacity to stimulate APC was evaluated by the production of IL-1 mediated by 7.0.3 either in iscom form (containing influenza antigens), or in matrix form or as free component. Iscom was the most efficient form. The matrix and free components also stimulated IL-1 secretion but at lower level and higher concentrations were required. The in vitro results were confirmed in vivo experiments. A significant proportion of IL-1+ splenocytes were detected by FACS in mice following treatment with iscoms.
CD30 HAS A SUSTAINED EXPRESSION IN TH2 CELLS

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CD30 is a 120 kDa surface antigen expressed by Reed Sternberg cells of Hodgkin’s disease, as well as by activated B and T lymphocytes. The immunologic function of CD30 is not yet determined. It is known, however, that 85 % of the CD30+ T cells co-express the T-helper (Th) marker CD4. To investigate whether CD30 defines a subtype of T helper cells, seven *Pityrosporum orbiculare* specific CD4+ T cell clones from a patient with atopic dermatitis were assessed for CD30 surface and gene expression. The clones were activated with OKT3 (anti-CD3 antibodies) and defined as Th1 (n=2), Th0 (n=2), and Th2 (n=3), according to their cytokine mRNA production detected by RT-PCR. OKT3 activation induced CD30 expression on more than 85% of the cells in all clones within one day as measured with flow cytometry using fluorescein-conjugated anti-CD30 (Ber-H2, Dakopatts, Glostrup, Denmark). A difference between the clones was noted in that the Th2 clones remained highly positive in CD30 reactivity whereas the other clones started to decline from day 3. Fourteen days after stimulation the Th2 clones expressed CD30 on 69 % of the cells, the Th0 clones on 15 % and the Th1 clones on 6 % of the cells. Still, 24 days after stimulation, we determined approximately 40 % CD30+ cells among the Th2 clones but only 5% or less in the Th0 and Th1 clones. The gene expression for CD30 as detected by RT-PCR correlated well to the cell surface expression of CD30. These data indicate that CD30 identifies activated CD4+ T cells and is sustained expressed in Th2 cells.

MUCOSAL AND SERUM ANTIBODY RESPONSES TO DEXTRAN-CHOLERA TOXIN B SUBUNIT CONJUGATES.

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After preparing and characterizing dextran-cholera toxin B subunit (CTB) conjugates, we studied their immunogenicity in mice following systemic or mucosal immunizations. Dextran of different molecular weights were conjugated via adipic acid hydrazide and N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP) to CTB. Intranasal immunizations with conjugates evoked local IgA antibody responses against dextran in the lung and peroral immunizations did the same in the small intestine. Intranasal immunizations also elicited serum antibody titers that were significantly higher or equal to those after subcutaneous immunizations. The antibody responses were dose-dependent. We have also studied the effect of preexisting antibodies to CTB on the magnitude of the anti-dextran response. The results show that it is possible to evoke a local as well as systemic antibody response against a polysaccharide by conjugating it to CTB and using an appropriate route of immunization.

CA2⁺ MOBILIZATION IN PHYSIOLOGICALLY STIMULATED SINGLE T CELLS CORRELATES WITH T CELL RECEPTOR SURFACE EXPRESSION.

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We have investigated the relationship between TcR expression and Ca²⁺ signaling. Intracellular free Ca²⁺ concentration ([Ca²⁺]) was monitored, by use of an imaging system, in single Fura-2 loaded CD4+ T cells stimulated with their physiological ligand, i.e. antigenic peptide bound to MHC molecules on antigen-presenting cells. 6 Th1 clones derived from mice transgenic for the αβ TcR (Vα1, Jα19; Vγ8.2, Dγ1, Jγ1.2) of the CD4⁺ T cells stimulated with their physiological ligand, i.e. antigenic peptide bound to MHC molecules on antigen-presenting cells. The specificity of the transgenic receptor is towards the 91-101 fragment of the MOPC315 λ2 L-chain, presented on the MHC class II I-Eᵇ molecule. The different Th1 clones express varying levels of the transgenic TcR as detected by staining with the clonotype-specific mAb GB113. Such non-homogenous expression of the transgenic TcR among the clones is due to endogenously rearranged TcR genes; i.e., most of the clones express two distinct TcR’s. The relative intensity of staining of the Th1 clones varied from 56 % (3G11, a clone also expressing TcR Vγ8.2) to 100 %, compared to 4B2A1 (the donor clone of the transgenes, expressing only one receptor). Both the maximum and mean [Ca²⁺] increase evoked in the various cloned T cells by 91-101(A2³⁵²³)-pulsed, I-Eᵇ expressing L cell fibroblasts correlated linearly with the relative intensity of GB113 staining. This indicates that quantitative differences in TcR levels may be an important factor in T cell activation.

MHC-I ligaton induces proliferaton and functional maturity in T-cells.

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The effect of MHC-I ligaton in human T-cells was investigated using immobilized anti-MHC-I mAb as a modelsystem. Our data shows that MHC-I ligaton induces proliferation in PBMC which is co-stimulated by CD28 ligaton. MHC-I induced proliferation in T-cells is dependent cellular interactions with non-T-cell. Exposure of PBMC for 24 hours to anti-MHC-I mAb and subsequent culture without stimulation induces proliferation and development of cytotoxicity in human T-cells. Functional maturity is accompanied by anti-MHC-I mAb induced upregulation of the TCR and CD28 molecules on T-cells. The upregulation of the CD28 molecule is greatly enhanced by simultaneous ligaton of the TCR. These results clearly demonstrate that MHC-I complexes may be important inducers of positive signaling in human T-cells. This is the first report on a functional relationship between the MHC-I complex and the CD28 receptor. The results also suggest that when T-cells act as APC they may become activated upon contact with an antigen specific T-cell.
In order to study factors contributing to septic arthritis we have analyzed the influence of bacterial superantigen production as well as T lymphocyte participation in the course of S. aureus arthritis.

**Methods:** Healthy Sprague-Dawley rats were inoculated i.v. with 5 superantigen producing S. aureus strains and one non-superantigen producing S. aureus control strain. The animals were assessed with regard to clinical course, and serum levels of IL-6, immunoglobulins and rheumatoid factors (Exp I). Rats inoculated with S. aureus AB-1 producing staphylococcal enterotoxin A were further analyzed by the use of histopathology and immunohistochemistry (Exp II). Furthermore, the clinical course was assessed in rats treated with anti-TCR monoclonal antibody R73 in connection with inoculation with S. aureus AB-1 (Exp III).

**Results:** Superantigen producing S. aureus strains induced arthritis in almost all rats, whereas the non-superantigen producing control strain only induced mild transient arthritis in 20% of rats. Serum IL-6 levels increased significantly in rats inoculated with superantigen producing S. aureus. In contrast, irrespective of staphylococcal strain used, there was similar polyclonal B cell activation. Histopathology showed erosive arthritis in a majority of rats within 11 days. Staining of arthritic joints revealed that 12% of infiltrating cells were CD4+ cells, many of which were activated. Blocking of the TCR led to a milder course of arthritis.

**Conclusions:** Polyclonal B cell activation does not seem to correlate to neither arthritis nor superantigen production. Bacterial superantigen production is a virulence factor in septic arthritis possibly through stimulation of T cells. T lymphocytes actively participate in the development of arthritic lesions.

**HUMAN ANTIPORCINE CYTOTOXIC CELLS.**

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Knowledge on how the human body defends itself against cells from other species is becoming increasingly important. The reaction of the human immune system towards porcine cells is interesting, because the swine might be our exit from shortage of organs for transplantation.

The purpose of our investigation is to characterize human antiporcine cells mediating cytotoxicity without being primed in vitro. In 3Cr-release assays, mononuclear cells of five of six healthy humans are able to lyse PHA-lymphoblasts from every swine tested (seven or eight). None of the six humans are able to lyse porcine mononuclear cells, from which the PHA-lymphoblasts are generated.

The cytotoxicity of human mononuclear cells to porcine PHA-lymphoblasts is blocked when unlabelled K562 is added, but K562 does not block as well as unlabelled porcine PHA-lymphoblasts, which implies that some antiporcine cells possess NK-activity.

To get further information on these cells, we do limiting dilution analyses, and clone the xenoreactive cytotoxic cells. Cloned cells are analysed as to phenotype, function and specificity.
Protein phosphatases PP1/PP2A play a critical role in interleukin-2 induced, LFA-1 dependent, homotypic adhesion in human CD4 T cell lines.

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As a growth factor for T lymphocytes, interleukin-2 (IL-2) induces LFA-1 mediated adhesion, migration, and extravasation of T lymphocytes. It is, however, largely unknown, how IL-2 receptors (IL-2R) are coupled to the LFA-1 adhesion pathway. Here, we show that calycin A, an inhibitor of protein phosphatases PP1/2A, strongly inhibited IL-2 induced proliferation and homotypic aggregation in CD4 T cells. In contrast, ligand induced downregulation of IL-2 receptors was not inhibited. In vitro assays, calycin A inhibited PP1/2A-mediated dephosphorylation of phosphorylase A, a physiological substrate for PP1/2A phosphatases. Likewise, in vivo pretreatment with calycin A inhibited PP1/2A phosphatase activity by approximately 70 %. Inhibitors of other protein phosphatases, such as cyclosporin A, had no effect on IL-2 induced homotypic adhesion or proliferating. In contrast, cyclosporin A almost completely blocked CD3 mediated proliferation in parallel experiments. In conclusion, we provide evidence that protein phosphatases PP1/2A play a critical role in IL-2 induced, LFA-1 dependent adhesion.

EXPANSION OF Vγ1/Vγ8 EXPRESSING AND Vγ DUAL EXPRESSING γδ T CELLS IN PERIPHERAL BLOOD OF PATIENTS WITH INFLAMMATORY BOWEL DISEASE

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In the present study, the Vγ and Vδ segment usage of γδ T cells in PB and among intraepithelial lymphocytes (IEL) of non-inflamed and inflamed intestine of patients with inflammatory bowel disease (IBD) was analyzed. Cell surface expression of the protein and gene transcription was assayed by using flow cytometry using a panel of V gene subtype specific mAb and PCR analysis using V gene specific primers. The Vγ T cell receptor (TCR) expressing cells were increased in PB of patients with Crohn's disease (CrD), but not in patients with ulcerative colitis (UC). In patients with UC, higher frequency of Vδ T cells was found in the non-inflamed than in the inflamed intestine. In IBD patients, the Vδ1 subset was clearly dominant among all γδ T cells in the intestine both as analyzed by flow cytometry. In conclusion, we provide evidence that protein phosphatases PP1/2A play a critical role in IL-2 induced, LFA-1 dependent adhesion.

In vitro secretion of Interleukin-4 and Interferon-γ in response to specific allergens in peripheral mononuclear cells from atopic and non-atopic individuals.

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CD4+ T cells can be divided into two major subsets, T helper (Th)1 and Th2 cells. Interleukin-4 (IL-4) is produced by Th2 cells and induces switching to Immunoglobulin E which induces histamine release from mast cells in allergy. Interferon-γ (IFN-γ) produced by Th1 cells counteracts the effects of IL-4. In this study we wanted to investigate whether the elevated specific IgE-levels in allergic patients were reflected by an increased number of IL-4 producing cells in response to allergens. The number of IL-4 and IFN-γ producing cells (ELISPOT) in response to specific allergens (birch pollen and hair from cat) in peripheral mononuclear cells from atopic and healthy individuals was compared. In the atopic donors there was an increase in the number of IL-4 producing cells in response to specific antigen as compared to healthy donors (p<0.001). When the allergen was combined with a suboptimal dose of PHA, there was a synergized increase in the number of IL-4 producing cells in the atopic donors, which was not seen with the number of IFN-γ producing cells. In conclusion, these data indicate that the elevated IgE levels in atopic are reflected by an increased number of allergen specific IL-4 producing cells.
The aim of the present study was to investigate the immune response in rats orally fed ovalbumin (OVA) compared to control rats fed a normal diet after immunization with OVA and an orally challenged with a bacterium producing OVA. Rats were made partially tolerant by feeding an OVA-containing diet from weaning for one to four consecutive weeks. Controls were weaned onto a standard diet without OVA. One week after ending OVA feeding, the animals were immunized with a mixture of OVA and human serum albumin (HSA) in Freund’s complete adjuvant (FCA) at one site of the back. Blood was collected two weeks later and the delayed type hypersensitivity (DTH) response was evaluated by challenging the rats with OVA and HSA in the ears. Five days thereafter the animals were then orally challenged by colonizing with an Escherichia coli producing OVA. All rats fed OVA showed a significantly suppressed DTH response to OVA compared to the rats fed standard diet. Furthermore only the rats fed OVA, showed bystander tolerance i.e. had a significantly lower DTH response to HSA than the controls fed a normal diet. Immunohistochecmistry of sections from the intestine showed a significantly higher IL-2 receptor expression, mainly in goblet cells and greater MHC Class II expression both in the villous core and epithelium in the controls than in the rats fed OVA.

Results from the present study showed that rats partially tolerant to one antigen can show a suppressed T-cell response to an unrelated antigen given simultaneously. This also leads to a reduced local inflammatory response when colonized with a bacterium producing OVA.

CHARACTERIZATION OF TWO DISTINCT MHC CLASS II BINDING SITES IN THE SUPERANTIGEN STAPHYLOCOCCAL ENTEROTOXIN

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Bacterial superantigens (SAg) are potent activators of T lymphocytes. To characterize potential MHC Class II binding sites in the SAg staphylococcal enterotoxin A (SEA) we performed alanine substitution mutagenesis throughout the C-terminal part of and at selected sites in the N-terminal domain. Three C-terminal amino acids, H225, D227 and H187, had a major influence on MHC Class II binding and appeared to be involved in coordination of a Zn ion. Mutations at H225 and D227 resulted in more than a thousand-fold loss of MHC Class II binding. Mutation at F47, which represents a N-terminal region homologous to the MHC Class II binding site previously demonstrated in the SAg SEB, resulted in 10-fold reduction of antigen given simultaneously. This also leads to a reduced local inflammatory response when colonized with a bacterium producing OVA.

STRUCTURAL CHARACTERIZATION OF PEPTIDES BINDING SYNOVIAL FLUID ANTIBODIES OBTAINED FROM RHEUMATOID ARTHRITIS PATIENTS

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So far very little is known about the antibody specificities in synovial fluid (SF) from rheumatoid arthritis (RA) patients. This is due to the lack of information about the antigens responsible for the B cell activation. However, the discovery of the peptide phage libraries offers the possibility of defining antibody specificities whether or not the antigen is known. We have to analyze the antibody specificities, by bio-panning of a random nine amino acid peptide phage library with antibodies from the SF of RA patients. Sequence analysis of peptides displayed by phages that bound to the antibodies from SF and serum of one RA patient, indicated that B cell expansion in the SF most likely is due to a local and specific antigen stimulation. We also showed that an anti-IL-4 receptor antibody can block T cell expansion in SF of RA patients. Taken together, the present results indicate that the expansion of B cells in the SF synovium is a product of locally antigen driven selection. In addition our data opens the possibility for defining the entire sets of synovium antibody specificities as well as common SF specificities between RA patients.

TH2 IMMUNE RESPONSE in P.falciparum MALARIA

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Efficient production of protective anti-malaria antibodies requires an intact and functional T-cells system. In mice, regulatory CD4+ T-cells can be divided into two functionally distinct subpopulations denoted TH1 and TH2 cells based on the cytokine profiles they produce. In human, recent studies also suggested functional heterogeneity of CD4+ T-cells. TH1 cells produce IL-2 and IFN-γ and TH2 cells produce IL-4, IL-5 and IL-13. TH1 cells mediate DTH and are involved in inflammatory reactions, while TH2 cells regulate specific antibody production. We have reported earlier that, in individual donors, IL-4 secretion was not associated with either proliferation or IFN-Y production, but was well correlated with elevated concentrations of serum anti-malaria antibodies (IgG) to the same peptides used for T-cell activation.

Since IL-4 is an important factor involved in immunoglobulin class switching from IgM/IgG to IgE, it was of interest to study the role of IL-4 in the regulation of anti-malaria IgE production. For this purpose, two groups of donors were selected. One with high and the other with low serum anti-malaria IgE antibody levels. Cells from the donor group with high serum anti-malaria IgE antibodies produced significantly higher IL-4 in response to a recombinant P155/RESA and a crude P.falciparum extract. Similarly individuals from this group had a higher potential to produce IL-4. The levels of serum anti-malaria IgE antibodies were well correlated with the capacity of cells to produce IL-4. These findings suggest a regulatory role of IL-4 in the production of anti-malaria IgE antibodies. The role and biological significance of these antibodies in malaria are presently under investigation.
Comparison of the number of IL-4 and IFN-γ secreting cells in response to the malaria vaccine candidate antigen Pf155/RESA in two groups of naturally primed individuals living in a malaria endemic area in Burkina Faso.

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The enzyme-linked immunosorbent (ELISPot) assay was used to enumerate the number of IFN-γ and IL-4 producing cells after in vitro stimulation with the malaria antigen Pf155/RESA or synthetic peptides corresponding to its major T-cell epitopes. Two groups of naturally primed individuals living in rural areas of Burkina Faso were studied. The donors comprised one group of healthy (non-parasitic) mainly adult people and one parasitic mainly younger people. IL-4 producing cells were detected in response to PHA but no such cells were detected in response to the malarial antigens. The most frequent IFN-γ responses were seen with Pf155/RESA. Thus, after stimulation with this antigen 52% of the donors responded positively in the ELISpot assay, while only 27% responded to the synthetic peptides, suggesting that the r-Pf155/RESA contained T-cell epitopes not covered by the peptides used in this study. The number of IFN-γ producing cells in response to the malarial antigens did not differ between the two groups. However, IFN-γ levels found in sera from the parasitic individuals were significantly higher than in those from healthy donors. This latter finding and the lack of differences seen in the number of IFN-γ producing spots in the two groups indicate that IFN-γ producing cells may have sequestered to other organs in the parasitic group.

A MURINE DENDRITIC CELL LINE PRODUCES IFN-α/β UPON STIMULATION WITH VIRUS AND BACTERIA.

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The murine dendritic cell line D2SC/1, immortalized with retrovirus, was stimulated by the viruses Herpes simplex virus (HSV), Sendai virus (SV) and the bacteria E. coli and S. aureus Cowan 1 (SAC). All induced production of IFN-α and -β in the D2SC/1 cells, determined at 24 h by immunoassays. IFN-β levels were usually higher than IFN-α levels. The HSV was clearly the most efficient inducer. Precultivation of D2SC/1 cells with mGM-CSF (24 h) and mIFN-β (2 h) markedly enhanced IFN-α/β production, except for SV. The latter inducer therefore uses another mechanism than e.g. HSV. Cells containing IFN-α and -β mRNA at 6 h were identified by in situ hybridization with 35S-labelled cRNA probes. Most cells showed a relatively low and uniform IFN-α/β mRNA expression, but in frequent cells (<1/1000) a very high IFN-α/β mRNA expression was detected. Priming with GM-CSF and IFN-β increased IFN-α/β mRNA levels. The pattern of IFN-α/β responses in D2SC/1 cells resembles that of human and porcine Natural Interferon-α/β Producing (NIP) cells, which have many characteristics of dendritic cells. The fact that they both produce IFN-α/β when exposed to microorganisms further indicates a function of these cytokines early in immune responses. The D2SC/1 cells also offer unique possibilities to study the regulation of IFN-α/β responses.

ANTIBODIES ARE CAPABLE OF DIRECTING SUPERTANTIGEN-MEDIATED T CELL KILLING OF CHRONIC B-LYMPHOCYTIC LEUKEMIA CELLS.

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The bacterial superantigen Staphylococcal enterotoxin A (SEA) is a highly potent activator of cytotoxic T cells when presented on MHC class II molecules of target cells. Our earlier studies showed that such SEA-directed T cells efficiently killed chronic B-lymphocytic leukemia (B-CLL) cells. With the ultimate goal to replace the natural specificity of SEA for MHC class II molecules with the specificity of a monoclonal antibody (mAb), we initially made a mutated protein A-SEA (PA-SEAm) fusion protein with >100-fold reduced binding affinity for MHC class II compared to native SEA. The fusion protein was successfully used to direct T cells to B-CLL cells coated with different B lineage specific (CD19, 20) or associated (CD37, 40) mAbs. The PA-SEAm protein was 10-100-fold more potent against mAb coated compared to uncoated HLA class II+B-CLL cells. No correlation was seen between the amount of mAb bound to the cell surface and sensitivity to lysis. Pretreatment of B-CLL cells by phorbol ester increased their sensitivity, and lysis was dependent on ICAM-1 molecules. However, no preactivation of the target cells was needed when a cocktail of 2 or 4 mAbs was used. Circulating leukemia and spleen cells were equally well killed. We conclude that the natural target specificity of SEA - MHC class II can be reduced by mutagenesis and novel binding specificity can be introduced by linkage to tumor reactive mAbs. Our findings encourage the construction of recombinant SEA mutant fusion proteins for specific T cell therapy of hematopoetic tumors such as B-CLL.
POSSIBLE CROSS REACTION BETWEEN BORDETELLA PERTUSSIS AND BRANCHAMELLA CATARRHALIS, DETECTED WITH THE USE OF MONOCLONAL ANTIBODIES

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The preparation of IgG2a monoclonal antibodies B2G7 to B. pertussis antigen from strain 475 reacts with the component of whole-cell lysates of B. pertussis strain 475 and B. catarrhalis strain 36621c, having a molecular weight of ca. 65 kD in immunoblotting. In ELISA McAb B2G7 reacted with B. pertussis and B. catarrhalis whole-cell lysates and with Re-glycolipid of Salmonella minnesota strain Re 395; this reaction could be inhibited by adding B. catarrhalis LPS to the preparation of McAb. The results thus obtained suggest that McAb B2G7 were specific to LPS, particularly to epitopes associated with the core or lipid A part of LPS. The fact that McAb reacted with the 65 kD component of whole-cell lysate is indicative of the presence of the LPS-protein complex. Close relationship between B. pertussis and B. catarrhalis LPS can be supposed.

ORAL TOLERANCE IS STIMULATED IN CD8-/- AND IL-4-/- (TH2-/-) MICE.

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Oral tolerance has been associated with systemic suppression exerted by CD8+ cells as well as by CD4+ cells of the Th2-phenotype. We investigated whether the complete absence of CD8+ T cells or IL-4, responsible for Th2-differentiation, would affect the ability to respond with oral tolerance (OT) to fed protein antigens. Therefore wildtype and gene targeted CD8-/- or IL-4-/- mice were orally fed KLH and subsequently challenged systemically with antigen plus RIBI adjuvant. Interestingly, all three types of mice normal, CD8-/- and Th2-(IL-4-/-) deficient mice exhibited similar levels of systemic tolerance after antigen feeding as compared to orally PBS-treated control mice. Both splenic anti-KLH SFC and T cell IFN-y production were significantly reduced in orally fed mice of all strains irrespective of the core and lipid A part of LPS. The fact that McAb reacted with the 65 kD component of whole-cell lysate is indicative of the presence of the LPS-protein complex. Close relationship between B. pertussis and B. catarrhalis LPS can be supposed.

THE BINDING C-REACTIVE PROTEIN (CRP) WITH IMMUNOGLOBULIN G (IgG) AND SOME CHARACTERISTICS OF THIS INTERACTION.

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CRP is the prototypic acute-phase serum protein in man. Biological effects of CRP include its interactions with wide ligands spectrum including phosphorilcholine containing molecules, some sera proteins, nuclear material and so on. We have found that IgG immobilized on the solid phase binds soluble human CRP. The binding of CRP by IgG was relatively sensitive to ionic concentration, giving maximal at physiological NaCl concentrations and low pH and was calcium-independent. Dissociation constant of this interaction was about 5.9 x 10^-6 M. The interaction depended slightly on IgG subclasses and IgG species. The immune complexes of rabbit IgG antibodies to human albumin with this antigen immobilized on the solid phase also bound CRP. It is possible that all or most of the CRP-binding capacity was localized on Fe-fragment of IgG. That is confirmed by the following facts: 1) for Fab fragments bound CRP very slightly; and 2) interaction of IgG antibodies to albumin with this antigen was not inhibited by CRP. From the other hand soluble aggregated IgG inhibited binding CRP with solid phase immobilized IgG. Also CRP changed complement activation ability of aggregated IgG. It possible that CRP may be involved into immune complexes formation via IgG and change the immune complex metabolism.

Local cytokine production in the colon during colitis induced by dextran sulfate sodium (DSS) in immunodeficient mice. A. Grönberg, E. Landström, A-K. Brus, and L-G. Axelsson. Dept. of Pharmacology, Pharmacia AB, Pharmacia & Upjohn Uppsala, S-751 82 Uppsala, Sweden, Fax +46 18 166459.

Oral administration of DSS to mice induces a disease with clinical and histological features reminiscent of human ulcerative colitis. We have investigated the local production of cytokines during the development of DSS colitis in mouse strains representing different forms of immune dysfunction, such as athymic (nu/nu) mice and mice with severe combined immunodeficiency (SCID). DSS was administered in the drinking water to specific pathogen free CD-1(BR), CD-1 (BR) nu/nu and CB-17 SCID mice. The mice were killed after 7 days and their intestines were cut out. Colon length and fecal water content were recorded and the colon was cut into small pieces and placed in 2% saponin buffer for extraction of cytokines. The cytokine content in the extract was measured using ELISA methods specific for IFN-γ, IL-6 and TNF-α. Colitis in euthymic and athymic animals, presented as shortening of the colon and diarrhea, was associated with elevated cytokine production seen in immunocompetent mice. However, the elevated cytokine production seen in immunocompetent mice may contribute to the inflammatory process.
FOLLICLES ARE A MAJOR SITE FOR THE CLONAL EXPANSION OF T CELLS IN A PRIMARY RESPONSE.

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In B10A mice, the response to pigeon cytochrome c (PCC) is largely restricted to T cells expressing vα11/vβ3 TCR. Less than 1% of the vα11/vβ3 cells possess canonical sequences associated with response to PCC in vivo. We quantified the occurrence of vα11/vβ3 T cells within splenic compartments by double immunohistology, following PCC immunisation. The background level of vα11/vβ3 cells in follicles is insignificant, but comprises approximately 0.5% of the cells in the T zones. T cells expressing vα11/vβ3 start to accumulate 4 days after primary immunisation within both the germinal centre and mantle of secondary follicles. This specific follicular response continues for at least a further 10 days. Up to 10% of follicular T cells take up 5-Bromo-2'-Deoxyuridine following 2 hour pulse labelling, indicating that these cells are in active proliferation within the follicle. No changes in vα11/vβ3 T cell numbers occurred in follicles in response to chicken δ-globulin. In the T zones the number of vα11/vβ3 T cells rises transiently between days 4 and 7 after immunisation, then falls rapidly to levels not significantly different from those in non-immunised mice. At the peak of the T zone response the number of vα11/vβ3 cells is double that of background. The data indicate that the clonal expansion of antigen-specific T cells in the follicles is at least as important as that in T cell rich areas.

Surface display compared to periplasmic expression of a malarial antigen in Salmonella typhimurium and its implications on the immunogenicity.

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We have used two different expression systems for the production of an 80 aminoacid sequence from the C-terminus of the Plasmodium falciparum blood stage antigen Pf155/RESA in an attenuated Salmonella typhimurium Aro A vaccine strain. Upon expression, the malarial sequence is targeted either to the periplasm as a soluble fusion protein containing two IgG-binding domains from the staphylococcal protein A (ZZ) or to the bacterial surface, inserted into the outer membrane protein A (OMPA) derived from Escherichia coli and Shigella dysenteriae. Both ZZM3 and OMPAM3 were stably expressed in the periplasm or on the surface of Salmonella respectively, but the ZZ expression system yielded 100 times more malarial immunogen than the OMPA system. Live recombinant Salmonella expressing ZZM3 or OMPAM3 were used to immunize mice. The ZZM3 hybrid induced antibodies to M3, ZZ and to Pf155/RESA whereas, OMPAM3 induced similar levels of antibodies reactive to M3 but not to Pf155; nevertheless, both recombinants induced a memory response to M3 and to Pf155/RESA. The high levels of M3 produced by the ZZ system make it suitable for the expression of heterologous antigens in Salmonella. However, in spite of the quantitative difference in M3 expression, both the ZZ and OMPA carriers provided comparable immunogenicity to M3, indicating that the display of M3 on the surface of Salmonella is equally efficient in priming the immune system as is periplasmic targeting.

REGIONAL SPECIALIZATION AND EXTRATHYMIC MATURATION OF HUMAN INTESTINAL INTRA-EPITHELIAL LYMPHOCYTES.

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The phenotype of intraepithelial lymphocytes (IEL) in human jejunal, ileum and colon was studied in situ as well as after isolation. γδ T cells constituted on average 30% of IEL at all levels of the intestine. The majority of these γδ IEL were CD4-CD8- and preferentially expressed V8.1δ. In contrast, αβ IEL showed a large variation in frequency and phenotype between different gut levels. CD8+ αβ IEL dominated in jejunum while cells with the unusual T cell phenotype, CD4+CD8-TCRδ+, constituted a major population of colonic IEL. CD4+ αβ IEL were equally represented as a minor population, at all three levels of the gut. These data suggest that γδ IEL are involved in surveillance of the epithelial cells whereas αβ IEL may participate in immune responses to luminal antigens.

A small proportion of IEL with thymocyte-like phenotypes (CD1+ TCRζ/CD3-, CD2+ TCRζ/CD3- as well as CD1+ TCRζ+ and CD1+ TCRζ+ IEL) were detected in jejunum. Furthermore, expression of recombinant activating gene-1 (RAG-1) mRNA was detected in jejunal but not in colonic IEL as determined by RT-PCR. RAG-1 expression was confined to jejunal IEL with immature phenotypes (CD2+ TCRζ/CD3- and CD3+ TCRζ- IEL). This strongly suggests that human small intestinal epithelium is a site for extrathymic T cell maturation.
INTERLEUKIN-4 IS NOT A SELECTIVE INDUCER OF VCAM-1 ON MICROVASCULAR ENDOTHELIAL CELLS FROM THE HUMAN GUT.

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The role of infected monocytes in measles pathogenesis is still poorly understood. We studied measles virus (MV) infection in cells of the human myeloid lineage with different maturation stages, from human bone marrow granulocyte/macrophage progenitors to macrophages. MV was able to infect myeloid progenitors CFU-GM (granulocyte-macrophage colony forming units), monocytes and monocyte-derived macrophages. MV infection in granulocyte-macrophage colonies of progenitor cells was productive, unlike in monocytes and macrophages, which supported virus RNA and protein synthesis without releasing infectious virus. Stimulation of infected monocytes by interleukin-3 and/or granulocyte-macrophage colony stimulating factor was not sufficient to change a restricted infection to a productive one. Maturation of infected monocytes to macrophages did not enhance measles virus production either.

In contrast to the results obtained with mononuclear cells, human promyelocytic and promonocytic cell lines HL-60, U-937 and THP-1, each of which represents a different stage of maturation, supported virus replication, and TPA (12-O-tetradecanoyl-phorbol-13-acetate) -induced maturation of the cells to the macrophage-like did not markedly alter virus replication.

MEASLES VIRUS INFECTION IN CELLS OF THE MYELOID LINEAGE.

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Humans repeatedly infected with the malaria parasite Plasmodium falciparum have elevated serum levels of IgE. In order to investigate the mechanisms of IgE elevation in malaria we studied mice immunized with the rodent malaria Plasmodium chabaudi chabaudi for total IgE and IgE antimalarial antibodies. Hyperimmune mice had elevated levels of total IgE in their sera as compared to non-infected control mice. The levels of total IgE were comparable to those of mice repeatedly infected with thehelmith Schistosoma mansoni, known to give rise to strongly increased IgE production. Sera taken from mice 3 weeks after one infection with P.chabaudi showed almost no IgE-elevation, indicating that prolonged or repeated exposure to the parasite is necessary for the induction of an IgE response. When tested in immunoblotting and ELISA, the sera were found to contain IgE antibodies specific for a variety of P.chabaudi antigens. The results indicate that malaria infection is directly involved in the induction of enhanced IgE levels.

NO LINKAGE OR ASSOCIATION WITH THE MBP GENE IN MS.

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Myelin basic protein (MBP) is a candidate autoantigen in multiple sclerosis (MS). Analysis of a tetrarepeat sequence 1 Kb 5' of the MBP gene in Finnish MS patients has indicated both linkage and association with certain alleles of a 1.2 Kb PCR amplification fragment. However, these findings have not been confirmed in subsequent analyses of North American and British patients. Likewise, analysis of ILA genes in MS has shown linkage in Finland and close segregation in a few Swedish families in contrast with negative results in recent investigations elsewhere. This could indicate ethnic differences with between Nordic MS and Western European MS, i.e. a geographic genetic heterogeneity. Thus it is of interest to study the MBP gene in a MS population as similar as possible to the Finnish.

30 Swedish nuclear families with 2, 3 or 4 patients with MS were investigated for an 8 allele amplification fragment (length 220-248) of the tetrarepeat 5' of the MBP gene, as well as 100 patients with relapsing/remitting MS and 28 patients with primarily chronic progressive MS and 94 healthy controls. In this analysis, PCR primers were labeled with ^32P and products visualized by autoradiography. Two-focus linkage analysis failed to reveal any linkage in the investigated families, regardless of different models of inheritance, gene frequencies and liability classes. Best fit revealed a negative LOD-score. Neither were there any differences in the distribution of alleles between familial or sporadic cases, nor between clinical subgroups, and controls.

Our conclusion is that the MBP gene does not appear to influence the susceptibility to MS in Swedish patients. This is in contrast with Finnish patients but in good agreement with other ethnic groups of European origin. Unlike type 1 diabetes, where the gene coding for the candidate autoantigen insulin has been firmly linked to susceptibility, the MBP gene, the primary candidate autoantigen in MS, does not seem to have this role.
The restored proliferative response in mercury low-responder mice in vitro
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Abstract

In vitro mercury causes autoimmune disease in the susceptible mice and rats. However, it is still not known how mercury ions primarily activate the immune system. Previous studies from our lab have shown that in vitro mercuric chloride induces a high proliferative immune response in mercury-susceptible mice, i.e. A/SW and Balb/c mice, in which both CD4^+ and CD8^+ T cells were activated, while in resistant mice as DBA/2 mice, a low proliferative response was induced. We expanded the studies and found that in vitro mercury ions primarily activate the immune system. Previous studies in our lab have shown that nonresponsiveness is linked to the A locus of MHC class II. However, the region between the crossover points is large enough to accommodate other genes in addition to those encoding the A molecule. We have crossed C57BL/6 mice which are transgenic for either the Aa or the Ab gene of H-2k haplotype. The offspring was tested by Southern blot and expression of A^k was analyzed by flow cytometry. Double transgenic, double negative as well as (C57BL/6 x CBA)F1 mice (BCF1, H-2^b/k) were immunized with IgE and BSA-TNP. After 1 and 3 weeks, animals were bled and sera assayed in ELISA. Neither the double transgenic mice nor the control littermates could produce BSA-specific antibodies, whereas a 150-fold enhancement was detected in BCF1 animals already after 1 week. Thus, lack of IgE/CD23-mediated enhancement of the BSA-specific antibody response in H-2^b mice may not be due to the A molecules themselves, but to other gene products encoded nearby.

PNEUMOCOCCAL POLYSACCHARIDE PROTEIN-CONJUGATE VACCINE IN A MOUSE MODEL: IMMUNE RESPONSE AND PROTECTION.
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The purpose of this study was to establish whether a pneumococcal type 4 polysaccharide protein-conjugate vaccine (PS4TT) gives a better antibody response and protection than a conventional 23-valent polysaccharide vaccine (Pneumovax\(^1\)N). Two inbred strains of mice (BALB/cABom and C57BL/6J) and one outbred strain (Hsd:NIHS) were immunized with PS4TT or Pneumovax, and antibody levels to type 4 polysaccharide were analysed. Survival and bacteremia were determined after i.p. challenge with 100 LD\(^{50}\) of the highly virulent pneumococcus type 4. 7/10 NIHs mice and 3/10 C57BL/6J mice showed an IgG response to the conjugate, whereas no BALB/c responded. In contrast, all BALB/c mice developed an IgG response. Protection with the conjugate was 100\%, 89\% and 73\% in NIHs, C57BL/6J and BALB/c mice, and protection with Pneumovax was 80\%, 80\% and 33\% respectively. 

Conclusions: The mouse model allows distinction between different levels of protection. Generally, PS4TT induced a better immune response and protection than Pneumovax. There was a discrepancy in individual mice between antibody response and protection.

IL-10 AND T-CELL NON-RESPONSIVENESS IN ACUTE MALARIA.

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Acute P. falciparum is associated with a pronounced reduction in the ability of peripheral T cells to respond to antigenic stimulation in vitro, while the mechanism behind this T-cell dysfunction is not fully understood. One possible cause is in vitro production of the immunosuppressive cytokine IL-10, previously implicated in peripheral T-cell non-responsiveness in acute visceral leishmaniasis. In the present study we have examined the role of IL-10 in malaria-induced T-cell non-responsiveness. Our preliminary results indicate that:

1. In vitro proliferation can not be restored by addition of a blocking monoclonal antibody to IL-10.
2. No inverse relation between IL-10 production and proliferation in vitro can be detected.
3. Significant reduction of in vitro lymphoproliferation of healthy T cells requires much higher levels of IL-10 than those measured in patient T-cell supernatants.

These results indicate that the well-established in vitro non-responsiveness of peripheral T cells from acute P. falciparum malaria patients is unlikely to be due to IL-10 produced in vitro. Results of these and further experiments on the mechanisms in malaria-induced T-cell non-responsiveness will be discussed.
Psoriasis vulgaris has HLA associations. We have previously defined certain HLA risk haplotypes for psoriasis. The aim was now to see if the risk haplotypes correlate with the clinical disease.

The series consisted of 64 patients and the clinical parameters were age of onset, family history of psoriasis, arthritis and clinical treatment periods at hospital. The HLA risk haplotypes including Cw6, DR7 and DQA1*0201 have been found in 30% of the patients. The presence of these risk haplotypes correlated with earlier age of onset (P=0.027) and family history of psoriasis (P=0.022) but not with arthritis or the number of clinical treatment periods.

Conclusion: HLA haplotypes with Cw6, DR7 and DQA1*0201 increase the penetrance of psoriasis genes in the family. The risk haplotypes precipitate the onset of psoriasis at an earlier age.

Weak response of immunosuppressive cytokines (IL-10, IL-4 and TGF-β), cause severe protracted and relapsing experimental autoimmune encephalomyelitis (SPR-EAE) in DA rats. Issazadeh S, Mustafa M.I, Lorentzen J.C, Höjeborg B., Olsson T. Institute for Molecular Medicine, Karolinska Hospital, Karolinska Institute, Sweden. Fax: +46 8 7744822

DA rats immunized by syngenic spinal cord emulsified in FIA develop a severe protracted and relapsing type of EAE, a model for MS. Here we used an in situ hybridization to study the dynamics of cellular mRNA expression of cytokines in the central nervous system (CNS) and lymphoid organs of SPR-EAE in DA rats and acute monophasic EAE in Lewis rats. A number of proinflammatory cytokines: T helper (Th)-1 (interferon /IFN-γ, tumor necrosis factor/ TNF-β) and interleukin /IL-12, TNF-α, IL-1β and cytokolin and putative immunosuppressive cytokines: Th-2 (IL-4, IL-10) and transforming growth factor / TGF-β was investigated. A high number of cells expressing mRNA for IL-12, IFN-γ, TNF-α, IL-1β, TNF-β and cytokolin was detected before clinical signs of SPR-EAE and then dramatically increased at acute phase of SPR-EAE. All these cytokines were also seen augmented at relapses, furthermore IL-12 and TNF-β were found elevated even at remission. Interestingly all proinflammatory cytokines were also detected in high numbers in acute monophasic EAE at height of the disease. While TGF-β and IL-10 raised during recovery phase of acute monophasic EAE. In contrast these two cytokines as well as IL-4 were almost absent in SPR-EAE in remission. Thus we conclude that (i) Th1 cytokines, IL-12, TNF-α, IL-1β and cytokolin are related to initiation and effector phase of autoaggressive neuroinflammation in both monophasic and SPR-EAE. (ii) TGF-β and IL-10 are associated with disease recovery in acute monophasic EAE in Lewis rats. (iii) The weak response of TGF-β, IL-10 and IL-4 in DA rats may play important role in developing of relapsing course of EAE.

CHOLERA TOXIN AND ITS B SUBUNIT AS MUCOSAL IMMUNOGENS AND CARRIER/ADJUVANT MOLECULES FOR INDUCING GENITAL IMMUNITY

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There is a great interest in developing vaccines against sexually transmitted diseases (STD) e.g. infections caused by Chlamydia and HIV. This requires knowledge about how to stimulate and how to measure immunity in the genital mucosa. We chose cholera toxin (CT) and its B subunit (CTB) as model antigens since especially CT is known to elicit a strong immune response at different mucosal sites. CT/CTB is also interesting to use as a carrier/adjuvant to antigens chemically coupled to CTB. To find out how to immunise to get an optimal local IgA response we immunised mice by several different routes. The repeated local vaginal immunisations, alone or in conjunction with rectal immunisation, and the intranasal route seems to be the best way for inducing an IgA response in the genital mucosa. The responses were localised to the vagina and cervix, whereas responses in the uterus and the fallopian tubes were low or absent. Thus it seems that to achieve a local IgA antibody response in the genital tract repeated vaginal or intranasal immunisations have to be made. Based on this knowledge we have also coupled peptide antigens from Chlamydia and been able to induce significant specific IgA responses in the genital tract mucosa.
APO-1/Fas RECEPTOR EXPRESSION IN HUMAN AND EXPERIMENTAL SJÖGREN'S SYNDROME. R. Jonsson and K. Skarstein. Broegelmann Res Lab, Univ of Bergen, N-5021 Bergen, Norway. Fax: +4755974689.

Defective Fas-mediated apoptosis of the immune system may contribute to the pathogenesis and development of autoimmune diseases such as Sjögren’s syndrome (SS). With this background we have analyzed the expression of the Fas protein in salivary glands of human and murine SS with anti-Fas mAb and immunohistochemical technique (ABC-immunoperoxidase). Inflamed salivary gland tissue was obtained from patients with primary and secondary SS and from MRL/lpr mice. The human SS-tissue presented a very diversified Fas staining pattern. The distribution of Fas-positive cells among the infiltrating mononuclear cells was quite disparate ranging from very few but mostly ~30% of the MNC. In the human SS tissue staining was mostly confined to acinar and ductal epithelium, but also very intense in the interstitium indicating Fas expression. Despite mutation of the Fas gene in the MRL/lpr strain, there was an expression of the apoptosis-related Fas protein increasing with age in salivary glands of these mice (~1-3% of the MNC). Furthermore, specific antibody therapy against oligoclonally expanded TCR β families did not significantly influence the Fas expression. Overall, the Fas expression was much lower in the autoimmune mice than in humans. The pattern of Fas expression in glandular tissue in SS suggests involvement of this apoptosis-signalling receptor molecule in the pathogenesis and/or lymphoid cell accumulation.

CATECHOLAMINES ARE SYNTHESIZED BY MOUSE LYMPHOCYTES AND REGULATE FUNCTION OF THESE CELLS BY INDUCTION OF APOPTOSIS. E. Josefsson, J. Bergquist, R. Ekman, A. Tarkowski. Depts. of Clinical Immunology, Psychiatry & Neurochemistry, and Reumatology, Univ. of Göteborg, Sweden. FAX 46-31-826791.

The sympathetic nervous system has been shown to influence inflammation and immune responsiveness. The aim of this study was to investigate if lymphoid cells are able to produce catecholamines and if catecholamines, once secreted, influence the immune reactivity.

Methods: The dopamine (DA) and norepinephrine (NE) content in mouse spleen cells, peritoneal macrophages and T and B cell hybridomas was examined by capillary electrophoresis. The impact of L-DOPA, DA and NE on proliferation, measured as thymidine uptake, and differentiation, measured as cytokine and immunoglobulin production, was studied in vitro in mitogen driven cell cultures. Spleen cells were cultured with L-DOPA and catecholamines and induction of apoptosis was studied by propidium iodide labeling and FACS detection of hypodiploid cell nuclei.

Results: DA was detected in spleen cells, peritoneal macrophages, B cell hybridomas and 2 of 3 T cell hybridomas in concentrations varying between 7x10^-8 and 7x10^-11 mole dopamine/cell. Also NE was found in a T cell clone and splenocytes. L-DOPA, dopamine and norepinephrine dose-dependently inhibited Con A and LPS stimulated proliferation, immunoglobulin production and formation of IL-2, INF-γ and IL-6 by spleen cells. L-DOPA, DA and NE dose-dependently induced apoptosis of splenocytes.

Conclusion: Since lymphocytes a) produce DA and NE, b) are suppressed by catecholamines and c) express catecholamine receptors there are biological prerequisites of an autocrine regulation of the activity of lymphocytes by catecholamines.

THE EXPRESSED KAPPA IMMUNOGLOBULIN LIGHT CHAIN V GENES IN MONONUCLEAR CELLS FROM PERIPHERAL BLOOD. L. Juul, T. Barington, L. Hougs, A. Svejgaard and V. Andersen. Department of Clinical Immunology and Medical Department TTA, State University Hospital, Copenhagen, Denmark.

We have developed an unbiased single-sided specific PCR technique to study the expressed human kappa immunoglobulin light chain genes in mononuclear cells from peripheral blood (MNC). Approximately 50 gene products from a MNC pool from 11 individuals and 50 from a single individual were cloned from cDNA, sequenced, and assigned to the germline gene with the closest over-all homology. Some Vκ families and genes were found to be used more often and some less often than expected from their occurrence in the germ-line. Thus, family VκIII was found to be the most frequently expressed followed by VκI. The repertoire expressed in the pool was more versatile than in the single individual, but in both cases three germline genes (A27 (Hum κ327), L6 (Vκ4) and A3/A19) were dominating. Overall, approximately 80% of the sequences could be assigned to eight germline genes. The Jκ proximal Vκ genes was preferentially used. In the pool Jκ3 was expressed rarely. The sequences were very mutated with an average homology to the closest germline gene of only 93%. N-additions were found in about half of the sequences. Unexpected lengths of CDR1 were found in a few of the sequences assigned to germline genes belonging to family VκIII. Thus a sequence assigned to the L6 gene had five additional codons between codon 27 and 28.

Human Thymic Epithelial Cells present superantigens for humane thymocytes and CD4+ T cells.

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The role of cultured human thymic epithelial cells (TEC) as antigen-presenting cells was investigated with the use of superantigens (SAg), SEA and SEB. Coculture of TEC and mature CD4+ T cells showed that TEC are able to present both alloantigens and superantigens to the CD4+ T cells, as measured by increased proliferation of the T cells. The presentation is dependent on expression of MHC class II and possibly other costimulatory adhesion molecules on TEC. TEC express ICAM-1, LFA-3 and CD40. TEC themselves are not stimulated to increased proliferation, but production of cytokines (IL-1 and IL-6) from TEC enhances the CD4+ T cell response. TEC do not express B7-1 but show a low expression of B7-2 and the SAg presentation can be further increased by the addition of monoclonal antibody against CD28. TEC also present SAg to subpopulations of human thymocytes: CD4+8-+, CD4+ and CD3 positive thymocytes. Thus TEC act as antigen-presenting cells but less effective than professional antigen-presenting cells, due to the absent or weak expression of B7. This may support, that TEC are more important in positive than in negative selection.
EARLY EXPRESSION OF TGF-β AND REDUCED ENLARGEMENT OF DRAINING LYMPH NODES AFTER IMMUNISATION OF ORALLY TOLERISED RATS

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Oral tolerance was induced in Wistar rats by feeding ovalbumin (OvA)-containing pellets during 2 weeks. Two weeks after the end of feeding the rats were immunised with a mixture of OvA and human serum albumin (HSA) in Freund's complete adjuvant. Every fourth day between day 7 and day 26, the popliteal lymph nodes (draining the immunisation site) were removed. The enlargement of the lymph nodes, the number of CD4+ and CD8+ T-cells and the expression of TGF-β and IFN-γ in the lymph node was monitored. In a parallel set of rats, the specific antibody production was monitored for 18 weeks after immunisation.

In the OvA-fed rats there was no size-increase of the draining lymph nodes after immunisation, whereas the nodes of the control rats were heavily enlarged from day 13 to day 24 after immunisation. Furthermore, from day 7 to day 15 scattered TGF-β expressing cells were present in the T-cell area of the lymph nodes of OvA-fed rats only.

The anti-OvA levels in sera from the rats revealed an initial B-cell priming of the OvA-fed group, with levels higher than the control group during the first to weeks. Thereafter, suppression governed the response, and from week 4 to 18 the anti-OvA levels were considerably lower than the controls. The suppression also affected the response to the bystander antigen HSA, as even the anti-HSA levels were lower in the OvA-fed group than in the control group.

HUMAN MUCOSAL IgA SUBCLASS RESPONSE IN RELATION TO INTESTINAL BACTERIAL FLORA

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The mucosal Ig response (numbers of IgA-, IgM- and IgG-producing immunocytes per defined mucosal length unit) as well as the local IgA subclass distribution were studied in by-passed jejunal segments with bacterial overgrowth and compared with normally functioning segments in adults. Sterile ileal urinary conduits were likewise examined by immunohistochemistry in children; the mucosa showed metaplasia and glandular atrophy, but the median numbers of IgA, IgM and IgG immunocytes per mucosal length unit only tended to be decreased. The jejunal segments with bacterial overgrowth showed minor histological changes and contained fairly normal numbers of IgA and IgG immunocytes, but there was a significant reduction (p<0.05) of IgM immunocytes (12 cells/length unit) compared with control mucosa (24 cells/length unit). The subclass distribution of IgA immunocytes showed for IgA2 that the median number per mucosal length unit was significantly decreased (p<0.01) in ileal conduits (7 cells or 30% of total IgA) but increased (p<0.05) in jejunal segments with bacterial overgrowth (42 cells or 43% of total IgA) compared with that in normal ileum (15 cells or 40% of total IgA) and jejunum (24 cells or 23% of total IgA), respectively. These data suggested an association between intestinal bacterial load and topical IgA subclass production.

PATTERN COMPLETION IN IMMUNE NETWORKS: SIMULATIONS BY A COMPUTER MODEL BASED ON CELLULAR AUTOMATA

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The application of parallel distributed processing to immune networks has first been introduced by Vertosick and Kelly [1]. By making use of Oudin-Cazenave enigma, the sharing of idiotypic determinants by antibodies directed to non-cross reacting epitopes [2], they further led to the concept of content-addressable immune memories [3]. This concept is especially interesting, since it explains the structure behind the ability of pattern completion abilities of the immune system. In this study, we develop further the model introduced by Seiden and Celada [4] in order to study the pattern storage capacities of the immune system. This model is based on a non-standard cellular automata. The results obtained shows clearly that the Oudin-Cazenave enigma enables the pattern completion ability of the immune system. In other words, it is possible that a secondary response towards a partly changed antigen (e.g. a mutated pathogen) is not altered, if these changes are under a certain limit.

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Antibodies to Re Glycolipid in Children with Chronic Pyelonephritis.

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The levels of IgM and IgG antibodies to Re glycolipid of Salmonella minnesota strain Re 395 were determined in the blood sera of 56 children aged 4-12 years with chronic pyelonephritis by enzyme immunoassay (EIA) techniques. In cases of the exacerbation of the disease the level of IgM antibodies was elevated in 3 out of 6 children and the level of IgG antibodies in 1 child. At the stage of incomplete remission the proportion of positive sera was 67% and 45% (6 out of 9 and 4 out of 9) respectively. During remission an elevated level of IgM antibodies was registered in 15 out of 40 sera (36%). The same number of sera had an elevated level of IgG antibodies. In the control group of children without any symptoms of infectious inflammatory processes no sera with elevated levels of IgG and IgM were detected. Thus, at the incomplete stage of remission patients were shown to yield positive reactions more often than at the period of exacerbation, while at the time of complete remission the number of patients yielding positive reactions decreased. It should be pointed out that at the stages of exacerbation and incomplete remission the number of sera with an elevated level of IgM antibodies considerably prevailed over the number of sera with an elevated level of IgG antibodies. The determination of the levels of IgM and IgG antibodies to Re glycolipid of Gram-negative bacteria was useful for the study of the specific features of serological reactions in renal and urinary tract diseases.

ACTIVITIES OF BRUSH-BORDER ENZYMES OF THE RAT SMALL INTESTINE IN GLIADIN-INDUCED ENTEROPATHY

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In the early postnatal development of rats fed artificial milk diet gliadin induces proliferation of intraepithelial and lamina propria lymphocytes similar that found in coeliac disease. To study the effect of gliadin on brush-border membrane enzyme activities the rat pups (inbred strain AVN) were given gliadin from day 7 and the third group, serving as control, received human albumin. On day 21, the rats were sacrificed and the activities of sucrase, lactase, glucoamylase and dipeptidyl peptidase IV (DPP IV) were determined in isolated brush-border membrane of jejunal and ileal enterocytes. The activity of lactase of jejunal and ileal brush borders of the first group was significantly decreased while that of the second group increased in comparison with the control. The activities of sucrase and glucoamylase were increased especially in the jejunum in both gliadin-treated groups. DPP IV activity markedly decreased in the ileum brush borders of the first group of rat pups. We conclude that gliadin-treatment does not negatively affect the expression of typical developmental and differentiation markers, such as sucrase and glucoamylase, during the first three weeks of postnatal life of the rat.
Number of tetanus toxoid specific antibody-, IFN-γ- and IL-4- secreting cells in response to tetanus toxoid in vivo.

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Tetanus toxoid (TT) is a potent immunogen which evokes strong antibody responses after immunization. Here, the numbers of anti-TT specific antibody-, IFN-γ and IL-4 producing cells in in vitro secondary responses to TT were estimated. Peripheral blood lymphocytes from healthy previously TT vaccinated individuals were activated with TT in vitro for different periods of time and the numbers of antibody- or cytokine-secreting cells were analysed by different ELISPOT assays.

The optimal concentration of TT for induction of both antibody- and cytokine secreting cells was 5 μg/ml. Kinetic analysis revealed an onset of antibody-producing cells around 96 hours with a peak at day six (144 hrs), while peak responses for cytokine-producing cells were seen after 48 hrs. Antibody- and cytokine secreting cells were frequently found even when the corresponding product was not detected in the supernatant by ELISA, indicating the higher sensitivity of the ELISPOT assay.

Both IFN-γ and IL-4-secreting cells were detected, with a numerical predominance of the former, suggesting a combined Th1 and Th2 response in individuals not recently exposed to TT.

A NOVEL COSTIMULATORY T CELL ANTIGEN COEXPRESSED ON RENAL CELL CARCINOMA.

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The A6H monoclonal antibody (mAb) raised primarily against human Renal cell carcinoma (RCC) has previously been shown to bind strongly to RCC, to a certain degree to colon carcinoma but only marginally to a variety of normal tissues. Immuno-histochemical analysis of RCC tissues, containing tumor infiltrating lymphocytes, revealed that A6H stained both tumor cells and lymphocytes. FACS analysis of human peripheral blood lymphocytes demonstrated that A6H mAb stained 85-90% of both CD4+ and CD8+ T cells, but not granulocytes, monocytes, NK-cells or B-cells. Furthermore 85-90% of naive and memory T helper cells were stained with A6H, suggesting that the A6H mAb defines unique subsets within these T cell populations. Dual staining showed that the A6H mAb bind to an antigen clearly distinct from a number of other cell surface molecules on T cells, including CD28, CD29, CD26, CD44 and ICAM-2. A6H mAb binding induced a second signal in anti-CD3 mAb activated T-cells, resulting in cell proliferation IL-2 receptor expression and vigourous production of IFN-γ and TNF and minor amounts of IL-2. Immunoprecipitation with A6H mAb indicated a molecular weight of 120-140.000 on both T cells and RCC. We suggest that the A6H mAb defines a unique T cell surface antigen, which is involved in signal transduction and is expressed on subsets of human T cells. The coexpression of A6H on T cells and tumor cells suggests a possible function related to common properties among these cells.

HLA-B27 INFLUENCES SURVIVAL OF SALMONELLA IN MONOCYTIC CELL LINE U-937

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To study the role of HLA-B27 antigen in interaction between monocytes and Salmonella, we used the monocytic cell line U-937 which was transfected with either HLA-B27 or HLA-A2 genes. The cells were first stimulated with phorbol ester and adherent cells were incubated with S. enteritidis for 1 hour. After different incubation times the cells were harvested. The number of intracellular bacteria was counted by colony forming units and bacterial structures were detected with specific antiserum by immunofluorescence technique.

The average number of living bacteria/cell was about 0.1 after 1 hour incubation. There was no remarkable difference between HLA-B27- or HLA A2-transfected cells. Bacteria/cell ratio was about 5-fold greater in HLA-B27-transfected cells than in control cells after 3 days of incubation. With immunofluorescence, at 1 day's incubation time about 90% of bacteria looked intact, but some processed particles were also seen. After 3-5 days of incubation most of the bacteria seemed to be in a processed form.

HLA-B27 does not influence the ingestion of S. enteritidis to U-937 cells. Salmonella can survive inside monocytes at least for 7 days. On the other hand killing and processing of bacteria begins immediately. HLA-B27 seems to modulate surviving of Salmonella intracellularly. This might be due to increased intracellular multiplication of bacteria or inefficient killing of bacteria by HLA-B27-positive U-937 cells.

THE EFFECT OF THE LEISHMANIA MAJOR SURFACE PROTEASE GP63 ON T CELL ACTIVATION.

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The Leishmania parasite is an intracellular pathogen living inside human macrophages. The most abundant protein on the surface of Leishmania promastigotes is the protease GP63. We have previously shown that T cells incubated with GP63 lose their ability to bind a panel of monoclonal antibodies directed against the CD4 antigen. CD4 is an important costimulatory molecule present on the surface of a subset of T cells. In this study we investigate whether this cleavage has a detrimental effect on the T cell function. Human T cell clones or PBMCs were preincubated with GP63 (0-100 μg/ml) and added to antigen pulsed antigen presenting cells, when after proliferation, cytotoxicity, and expression of cell surface molecules were measured. Surprisingly, the proliferative response of antigen stimulated T cells treated with GP63 was equivalent to that of untreated cells. We have analyzed the CD4 molecule of cells treated with GP63 using SDS-page and western blot. Results from these and other experiments will be presented.
COMPLEMENT REGULATORY PROTEINS IN SALIVA AND SALIVARY GLANDS OF SJÖGREN’S SYNDROME PATIENTS

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Membrane cofactor protein (MCP, CD46), decay-accelerating factor (DAF, CD55), protecin (CD59) and clusterin (SP40,40) are complement regulatory proteins with wide tissue distribution. In this study the presence and localization of these glycoproteins were investigated with respect to their roles in the protection of cells in exocrine glands from complement attack. Whole saliva and salivary gland tissues of patients with primary Sjögren’s syndrome (SS) were compared with similar specimens from control subjects. The presence of the soluble form of these proteins in saliva specimens was detected by Western blot and dot blot techniques. Expression of these proteins in tissue was assessed by immunoperoxidase staining. CD55, CD59 and SP40,40 were detected in saliva samples, and their estimated molecular mass was in the range previously reported. CD46 was not detectable in saliva. With respect to salivary gland specimens, staining was more widely distributed in inflamed salivary gland tissue than in normal tissue. Immunostaining of CD46, CD59 and SP40,40 was strongly positive, but staining of CD55 was weak. For normal specimens, staining was mainly confined to the luminal surface, in ductal walls, and in connective tissue. In the inflamed salivary glands these proteins were intensely expressed on acinar and ductal epithelium, with a high proportion of positive mononuclear lymphoid cells in the foci. These findings suggest that SS tissue exhibit enhanced complement activation. Expression of these proteins may be an important determinant of the severity of tissue injury produced by complement-fixing salivary gland (auto)antibodies in SS.

Comparison of normal human B cells and the lymphoblastoid B cell-line, RAJI, as activators of the complement cascade

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Comparison was made of the deposition of anti-C3c- and anti-C3d-reactive fragments on normal B cells and the B cell-line, RAJI, after exposure to normal human serum in the absence and presence of 5 mM MgCl2/20 mM EGTA, to block the Ca2+-dependent classical activation pathway.

RAJI cells were observed to bind more than 10-fold greater amounts of anti-C3c and five-fold more anti-C3d than normal B cells. The difference in the ratio of reactivity of the serum-treated RAJI- and B cells with anti-C3c and anti-C3d (1.49 ± 0.48:1 and 0.71 ± 0.14:1, respectively) was significant, indicating that a substantially higher proportion of the C3 fragments deposited on B cells were degraded to the terminal, C3dg, product. In addition, complement deposition on RAJI cells displayed a greater Ca2+-dependency than the deposition on B cells.

Measurement of C3 fragment receptors revealed that the RAJI cells expressed ca. 5 times as many CR2 and 10-fold fewer CR1 than normal B cells. The implications of these findings will be discussed.

Antigen presentation studies of a T cell epitope inserted at different positions in an antibody heavy chain

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This project is based on a well-studied interaction of a particular T cell receptor which is specific for a peptide (91-101(l2315)) bound to MHC class II (I-E"). The l2315 peptide constitutes one of the antigen binding loops (CDR3) of an antibody light chain, and loops corresponding to the CDRs are present in all Ab domains. To study how antigen presentation of the peptide is dependent on its position in different protein contexts, we used in vitro mutagenesis to move the epitope to loops in the human IgG3 heavy chain. Three different mutants were made, each with one of the three CDR-corresponding loops of the C61 domain replaced with the l2315 peptide. These mutants were transfected into an I-E"-positive fibroblast cell line, and the resulting clones assayed for ability to stimulate l2315 specific T cells. It appears that the mutated heavy chains are retained intracellularly in the transfected fibroblasts, nevertheless, presentation on class II is obtained. We observe different efficiencies of antigen presentation when the epitope is inserted in different positions in the heavy chain.
THE CYTOKINE PROFILE OF HUMAN INTESTINAL INTRAEPITHELIAL LYMPHOCYTES SUGGESTS TH1 AND CYTOTOXIC FUNCTIONS.

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Cytokine mRNA expression in freshly isolated intraepithelial lymphocytes (IEL), in in vitro stimulated IEL and in subfractionated IEL was determined using RT-PCR. Freshly isolated IEL from different levels of the intestine showed strikingly similar cytokine profiles. Thus, IEL from jejunum, ileum and colon expressed mRNA for CD45RO as well as for IL-1β, IL-2, IL-8, IFN-γ and TNF-α. IEL from ileum also expressed TGF-β1. In in vitro activation of IEL from small intestine induced expression of IL-10, TFN-β, TGF-β1, IL-2, IFN-γ and TNF-α. Taken together the cytokine profile suggests that IEL are involved in cell mediated cytotoxicity and suppressor functions in vivo. Cytokine analysis of subfractionated IEL showed that the three major subpopulations, TCRγδ+, TCRββ+ and TCR-CD1+ IEL, all expressed IL-2, IFN-γ and TNF-α which is consistent with TH1 and/or cytotoxic effector functions. IL-1β and IL-8 were exclusively expressed in TCR-CD1+ cells.

Intestinal epithelial cells in jejunum but not in colon expressed HLA-DR and hsp60 as determined by immunoflow cytometry. Small intestinal epithelial cells may therefore function as antigen presenting cells. However, they only expressed mRNA for IL-1β, IL-8 and occasionally TNF-α suggesting that antigen presentation by epithelial cells may lead to anergy in IEL.

DIFFERENCES IN THE IMMUNE RESPONSE TO A PNEUMOCOCCAL POLYSACCHARIDE VACCINE IN TWO INBRED AND AN OUTBRED STRAIN OF MICE.

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Genetically determined factors may be important for the response to vaccines. Outbred mice do not exhibit the extent of genetic variation found in a human population. Inbred strains of mice may show large, genetically determined differences in their immune response. As part of the Dutch-Nordic Vaccine Consortium’s efforts to develop a new pneumococcal polysaccharide-protein conjugate vaccine, we studied the response to s.c. immunization with a 23-valent pneumococcal polysaccharide vaccine (Pneumovax®N) in two inbred strains of mice, BALB/cABom and C57BL/6J/Bom, and in outbred Hsd:NHS mice. The IgM antibody response 6 days after vaccination measured in an ELISA assay with the vaccine as antigen, was largest in BALB/c mice and lowest in C57BL/6J mice. After 13 and 30 days, BALB/c and NHS mice showed similar antibody levels, whereas C57BL/6J mice continued to show a lower response. The outbred NHS mice showed great variation in their response serotype 6B. BALB/c mice showed a faster response to this serotype after vaccination than C57BL/6J mice. The response to three other serotypes, 14, 19F and 23F, was virtually absent in all the mouse strains. Low levels of IgG antibodies were detected only in some NHS mice. Thus, BALB/c, C57BL/6J, and NHS mice differed markedly in their response to a conventional polysaccharide vaccine.

ESTABLISHMENT OF IN VITRO SYSTEMS TO STUDY THE ACQUISITION OF PERIPHERAL TOLERANCE.

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Acquisition of peripheral tolerance is unclear but at the T cell level, anergy has been suggested as the main mechanism of peripheral tolerance. Anergy is attained by viable cells after exposure to an appropriate signal. These cells are thought not to be deleted but are rendered anergic upon stimulation with the same signal. Many experiments studying peripheral tolerance have been carried out in vivo. In our laboratory two methods are carried out to study this mechanism. In vitro. Both methods are based on providing a negative signal to the murine T cell receptor region(TCR), Vβ8. After receiving a subsequent second signal from a polyclonal activator like Con A, the Vβ8 family would not increase in numbers despite the presence of other transformed and proliferating T cells. The importance of the second signal is to induce proliferation because prolonged stimulation with the first signal appears to result in cell death. TCR monoclonal antibody, F23.1 (anti-Vβ8,1,2,3) and a superantigen, Staphylococcal Enterotoxin B, (SEB), were used to provide this negative signal to the Vβ8 region of the TCR. In the first system, cells were cultured with either the soluble or immobilised form of F23.1 monoclonal antibody. The soluble form of F23.1, significantly reduced the Con A induced proliferation. In the second system, SEB was cultured with pure T cells (>80% purity) O/N then re cultured with Con A. Reduction in T cell proliferation with Con A stimulation can be seen in purified T cell cultures (day 1 to 2 but not on day 3). The presence of remaining APCs might cause interference at the presentation level i.e. SEB would be presented to T cells leading to proliferation instead of anergy. The success of these methods would allow an extensive study of peripheral tolerance in vitro for the first time.

The c-myc protein represses the 5′ initiator

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The 5′ gene is expressed only in pre-B cells. The 5′ region of the 5′ gene can be divided into 2 regions; B15, which allows expression only in pre-B cells and A5 which acts as promoter, contains all transcription initiation sites and is active in all cell types. Two A5-derived oligonucleotides (InrA5:1 and InrA5:2), each with a transcription start site, promote transcription in transient transfection assays, while a third oligonucleotide (90A5), without a transcription initiation site, is inactive. The InrA5:1 and InrA5:2 oligonucleotides, but not 90A5, form a DNA-protein complex containing c-myc and myn (murine homologue of Max) in gel retardation analysis. The myc and myn/Max proteins bind also to several other initiator-like sequences present in genes expressed early in B cell development. Transient transfection analysis suggest that, in this system, transcription depends on a transcription initiation site and appropriate flanking sequences. The significance of c-myc binding to these initiators is not clear, however, overexpression of c-myc in co-transfection assays represses the transcriptional activity of the 5′ initiator.
Cellular immunity in cynomolgus monkeys rectally exposed to a low dose of SIVSM
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Objective: To characterize the virus specific immunity, especially the cell-mediated immunity in monkeys which received a subinfectious dose of a macaque cell grown stock of SIVSM (SMM-3).

Methods: Cynomolgus monkeys were inoculated with a SIVSM stock propagated in vitro on cynomolgus monkeys PBMCs. Tenfold serial dilutions of stock virus ranging from 10⁻¹ to 10⁻³ was attained. The inoculated animals (IR) in 6 monkeys (2 animals for each dilution). Infection was monitored by virus isolation and PCR(gag, pol, env, LTR). Virus specific immunity was investigated by ELISA, Western blot, T-cell proliferation(HIV-2 viral lysate, SIV env synthetic peptides) and CTL(gag/pol, nef).

Results: Infection was demonstrated by positive virus isolation and PCR and by seroconversion in two of two monkeys given the 10⁻¹ dilution and 1/2 became infected given the 10⁻² dilution. The remaining three IR inoculated animals did not become infected as shown by negative virus isolation, serology and PCR but showed SIV specific gag/pol and nef CTL and T-cell proliferation against SIV env synthetic peptides.

Conclusions: Monkeys exposed to low concentrations of SIV developed SIV specific CTL and T-cell proliferation but no demonstrable viral antibodies. These animals have recently been rechallenged with a higher dose to determine if the cellular immunity can confer protection against challenge.

BINDING TO WHOLE BLOOD CELLS OF IMMUNE COMPLEXES FORMED AND OPSONISED IN SITU.
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In previous studies in this laboratory preformed IC were employed in flowcytometrical analyses of the distribution of immune complexes (IC) on blood cells and the buffering capacity of erythrocytes (E). In an effort to approach more closely physiological conditions, we have chosen to examine the binding to whole blood cells of IC formed in situ from FITC-conjugated tetanus toxoid (TT) and natural anti-TT antibodies contained in the donor serum. To this end, the anti-TT levels of a range donors were assessed by ELISA, and selected donors were employed in the investigations.

The binding of IC formed in situ and preformed IC, incubated for 1 hr. at 37°C (both at a ratio corresponding to the equivalence point) to whole blood cells was compared. It was found that uptake by leukocytes was considerably delayed for the IC formed in situ. In contrast, maximal binding to E occurred at 3 min. for both types of IC. Shifting the ratio towards antigen- as well as antibody-excess retarded the time point of maximal binding to E of in situ formed IC. The mechanisms underlying these observations as well as capacity of E to restrict the uptake of in situ formed IC are currently under investigation.

COMPARISON OF LOCAL AND SYSTEMIC IMMUNE RESPONSES AGAINST PUTATIVE VIRULENCE FACTORS OF HELICOBACTER PYLORI IN PATIENTS WITH DUODENAL ULCERS AND IN ASYMPTOMATIC CARRIERS.
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Antibody responses against putative virulence factors of H. pylori were determined in sera and gastric aspirates from patients with duodenal ulcers (n=19), in asymptomatic H. pylori carriers (n=18) and in non-infected age-matched controls (n=20). This was done to evaluate if antibody specificities are found in asymptomatic carriers that are lacking in ulcer patients and if immune responses may be induced locally in the stomach but not in serum.

Antibody levels against H. pylori lipopolysaccharide, flagellin, urease and two surface antigens, i.e. a 26 and a 30 kDa protein, and for control purposes against whole membrane proteins (MP), were tested in gastric aspirates and sera by means of different ELISA methods. A majority of the infected subjects had significantly higher levels of specific antibodies against the flagellin, the urease and the MP in sera and in gastric aspirates than the healthy controls.

In no instance were antibody responses against any particular H. pylori antigen seen in the asymptomatic but not in the symptomatic H. pylori carriers. The antibody responses were generally more pronounced and more abundant in sera than in gastric aspirates.
MISMATCH OF CD8 EXPRESSION AND CLASS II-RESTRICTION IN T CELL RECEPTOR TRANSGENIC MICE DEPENDS UPON ENDOGENOUS TCRα CHAINS.

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CD4+ T cells usually recognize peptide bond to MHC class II molecules while CD8+ cells recognize peptide on class I molecules. Such a correlation between coreceptor expression and MHC-restriction is thought to be a consequence of positive selection in the thymus. Here we describe an exception to this rule. CD8+ T cells are surprisingly frequent in mice transgenic for an αβ TCR which recognize an Ig light chain (λ219) idiotypic peptide, presented on the I-Eβ class II molecule. These CD8+ cells are now demonstrated to be λ219-specific and I-Eβ class II-restricted. CD8 expression seems to be of no functional importance for the class-II restricted specificity. Most of the CD8+ cells express endogenous TCRα chains. Such endogenous α-chains appear to enhance positive selection because the CD8+ population is dramatically reduced in recombinant deficient TCR-transgenic SCID mice. The repertoire of endogenous TCRα chains in CD8+ cells is skewed, indicating that only certain Vα domains may augment positive selection. Expression of endogenous TCRα chains becomes more frequent as thymocytes make the transitions from CD4+CD8αβCD4′CD8− to CD4−CD8+αβCD8+. A mechanism for positive selection of T cells with two TCR, as well as a mismatch between coreceptor and MHC-restriction, is discussed. The CD8+ class II-restricted T cells kill LPS blasts from λ219-transgenic mice, suggesting that such T cells could function as idotype-specific suppressors of B cells.

TFNα dominates cytokine mRNA expression in lymphoid tissues of rats developing arthritis.

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Development of an inflammatory self-limiting polyarthritis can be provoked in DA rats by Freund's incomplete adjuvant (FIA). Rats from the same strain develop a more severe and chronic arthritis when rats type II collagen (RCII) is administered with FIA. If an irrelevant antigen is added to these arthritogens, in this case ovalbumin (Ova), the development of arthritis is completely inhibited. This inhibition is disease-specific and long-lasting. To investigate mechanisms responsible for induction, as well as inhibition of arthritis, a kinetic study of local cytokine production in lymph nodes has been performed in rats immunized with the above mentioned agents. By using in situ hybridization techniques, the mRNA expression of TNFα, IL2, IFNγ and IL4 was determined. A rapid and pronounced expression of TNFα mRNA was recorded in RCII/FIA and FIA immunized rats, already 2 hours after immunization. This pronounced expression was not observed in Ova/FIA injected animals, which instead were the only animals where the IL4 gene was expressed. The expression of IFNγ mRNA was limited in RCII/FIA and FIA-immunized rats, whereas IL2 mRNA expression was only detected after RCII/FIA injection. Lymph node cells from RCII-immunized animals generated a high amount of TNFα after restimulation with RCII, whereas restimulation with the mitogen Con A generated a cytokine mRNA response dominated by IL2 and IFNγ. These and other results indicate that a strong local expression of TNFα, induced by arthritogenic stimuli, may be important for the induction of arthritis. Moreover, the elicitation of an immune reaction to an irrelevant antigen, may inhibit arthritis development by modulating the arthritis associated cytokine response.
PROINFLAMMATORY AND IMMUNOSUPPRESSIVE CYTOKINES IN MULTIPLE SCLEROSIS

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The inflammatory nature of multiple sclerosis (MS) implicates the involvement of immunoregulatory cytokines. Evidence is accumulating that IFN-γ, TNF-α and TNF-β have disease-promoting properties in MS, while TGF-β, IL-10 and, probably, IL-4 may exert some protective effects. In the present study we used in situ hybridization with cDNA oligonucleotide probes to detect and enumerate mononuclear cells (MNC) expressing IFN-γ, TNF-α, TNF-β, TGF-β, IL-4 and IL-10 mRNA in blood and cerebrospinal fluid (CSF) from 77 patients with MS, 23 with optic neuritis (ON) and controls. The MS patients had elevated numbers of blood MNC expressing all 6 cytokines both when enumerated without previous culture and after culture with myelin basic protein (MBP). IFN-γ, TNF-α, TGF-β and IL-4 were further enriched in the CSF of MS patients. Numbers of TNF-α mRNA expressing blood MNC correlated positively with the severity and progression of MS. In contrast, TGF-β and IL-4 were significantly higher in patients with shorter duration and minor disability, suggesting a beneficial downregulating effect of IL-4 and TGF-β in MS. Patients with ON, in many instances representing early MS, had similarly augmented numbers of TNF-α, TNF-β and IL-10 positive blood MNC as clinically definite MS. On patients examined within one month after onset had lower numbers of MBP induced IL-10 mRNA expressing cells compared to remission, indicating a possible protective role of IL-10. The results suggest that IFN-γ, TNF-α, TNF-β, TGF-β, IL-4 and IL-10 are involved in MS. TNF-α could be useful as a disease activity marker in MS, and regimens promoting TGF-β and IL-10 could influence MS beneficially.

THE ROLE OF COMPLEMENT RECEPTOR, TYPE 1 (CR1, CD35) IN THE UPTAKE OF IMMUNE COMPLEXES BY BLOOD CELLS.

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Erythrocytes (E) express low numbers of complement receptor, type 1 (CR1, CD35), via which they bind opsonised immune complexes (IC) in competition with leukocytes expressing high numbers of CR1 as well as other complement- and Fc-receptors. We examined the cellular distribution on whole blood cells or isolated leukocytes of preformed tetanus toxoid (TT)/human anti-TT immune complexes, opsonised in situ in 80% serum.

Reflecting the kinetics of C3-fragment incorporation, E took up IC rapidly, accounting for the major proportion of the total early cellular IC-binding (median: 83% after 30 sec. and 92% after 3 min.). The presence of E reduced the leukocyte uptake of IC after 30 sec. by >60%. Subsequently, the IC-binding to E and the restrictive effect exerted by E decreased. After 15 min., E inhibited the IC-uptake by phagocytes by <20% and promoted a progressive uptake by B cells, which could be abolished by blockade of E-CR1 with the MoAb, 3D9. CR1-blockade inhibited the initial IC-uptake by phagocytes by >85%. and <45% after 15 min. Our findings support the hypothesis that E act as a buffer, restricting the IC-uptake by circulating phagocytes within the first minutes of incubation during which CR1 is the dominant receptor, and suggest that the presence of E enhances the late IC-uptake by B cells.

Cytokine profiles of microvascular endothelium from the human gut.

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Increasing evidence suggests that leukocyte emigration to areas of inflammation depends on endothelial cell activation, which may also result in production of important cytokines by such cells. These cytokines may further enhance the inflammatory process, but organ-specific differences must be taken into account. Methods: To evaluate cytokine profiles of putative importance for the development of inflammatory bowel disease, we examined human intestinal microvascular endothelial cell (HIMEC) mRNA expression for 12 cytokines with reverse transcriptase-PCR (RT-PCR). The cytokine profiles were compared with those obtained in human umbilical vein endothelial cells (HUVEC). Cells were cultured to confluence in 12-well plates and then stimulated for 4 h with rh IL-1β (100 U/ml) or rh IL-4 (100 U/ml). Different cytokine mRNAs were detected by cDNA synthesis and PCR amplification of polya RNA isolated from cultured cells. Results: Unstimulated HIMEC expressed low levels of mRNA for IL-1β, IL-6 and TNF-α. However, IL-1β, and to a lesser degree IL-4, increased mRNA for these three cytokines. We were unable to detect mRNA for IL-1α, IL-2, IL-3, IL-4, IL-5, IL-10 or IFN-γ in HIMEC. By comparison, in HUVEC IL-1β stimulated mRNA for IL-1α, IL-1β and IL-6, but not for the other cytokines. Message for TGF-β and IL-8 was present in both cell types and was not subjected to regulation by IL-1β and IL-4. Conclusion: Our results suggest that TNF-α expression is preferential for HIMEC and that both HIMEC and HUVEC produce IL-1β, IL-6, IL-8 and TGF-β. Some of these cytokines are known to be powerful inducers of endothelial cell adhesion molecules, and TNF-α may contribute to tissue damage in various gut diseases.

HUMORAL IMMUNE RESPONSE IN CYTOMEGALOVIRUS MACAQUES VACCINATED WITH NATIVE HIV-2 GP125

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OBJECTIVE: To characterize the antibody (ab) response in cynomolgus macaques immunized with native HIV-2 envelope glycoprotein gp125 coupled to immune stimulating complexes (iscoms) or Ribi adjuvant.

METHODS: Nine monkeys were immunized at 0, 2, 4, 6, and 9 months with HIV-2/SBL6669 gpl25, purified by affinity chromatography using Galanthus nivalis agglutinin. Five monkeys received 30 μg of gp125 in iscoms and four monkeys received 50 μg gp125 in Ribi adjuvant. Serum samples collected fourteen days after each immunization were analysed for ab against HIV-2/SBL6669, gpl25 and the V3 region of gpl25. Abs were determined using ELISA technique.

RESULTS AND CONCLUSION: Immunization with gp125 in iscoms resulted in maximum ab levels already after two immunizations and did not increase on boosting. In the gp125-Ribi immunized monkeys low ab titers were initially observed. On completion of the immunizations ab titers had reached levels comparable to those in the gp125-iscom immunized monkeys. Fourteen days after the final booster the monkeys were challenged with 30 MID50 of homologous HIV-2/SBL6669. No differences with respect to induction of protection was observed since two of five gp125-iscom immunized monkeys and two of four gp125-Ribi immunized monkeys were protected.
THE STAPHYLOCOCCAL ACCESSORY REGULATOR (SAR) CONTROLS STAPHYLOCOCCUS AUREUS VIRULENCE IN A MURINE ARTHRITIS MODEL

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*S. aureus* arthritis is a highly erosive disease where both host and bacterial determinants are of importance in its induction and progression. The aim of our study was to assess the role of sar gene as a virulence determinant in the pathogenesis of septic arthritis.

**Materials and methods.** *S. aureus* strain, isogenic for sar locus, was inoculated i.v. into Swiss mice. Clinical, bacteriological and histopathological progression of the disease was then studied.

**Results.** The peak frequency of arthritis within 1 week after inoculation of bacteria was 79% in the group of mice inoculated with wild, sar+, strain whereas corresponding frequency in sar- mutant was 21% (p<0.01). Also, infection with sar* staphylococci led to significantly higher weight loss. To assess the relationship between clinical signs and spread of bacteria we analyzed the early joining pattern and persistence of *S. aureus* in joints, kidneys, spleen, liver, and peripheral blood. Organs from sar+ inoculated subjects contained at later stages a considerably higher amounts of live bacteria.

**Conclusion.** Our results suggest that the sar system of *S. aureus* is an important virulence determinant in the induction and progression of septic arthritis.

ANTI-INFLAMMATORY PROPERTIES OF NK CELLS

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NK cells take part in innate immune responses by cytolytic of certain virally infected and tumor cells. They have also been ascribed a role in controlling myelopoiesis and lymphocyte development. We set out to investigate if NK cells have a role in controlling the intensity of in vivo inflammatory responses.

**Methods:** In outbred SWISS mice, NK cells were depleted by repeated intraperitoneal injections with an antibody to the NK1.1 antigen, derived from the mouse hybridoma PK136. Controls received an antibody to Herpes virus. Granulocyte mediated inflammation was induced by injection of 30 microliters of olive oil intradermally in the hind footpad. 24 hours later, swelling of the footpad was registered. In other experiments, mice were immunized and boosted with oxazolone to assess delayed type hypersensitivity (DTH).

**Results:** Granulocyte mediated inflammation was not significantly changed 24 hours after NK cell depletion. After 8 days, a significant (p < 0.05) increase of swelling was registered. Concomitantly, there was a tendency towards increased numbers of peripheral blood lymphocytes and granulocytes. In the DTH model, a tendency for increased reaction was seen in NK cell depleted mice.

**Conclusion:** Our results point to an important role for NK cells in regulating the intensity of inflammatory response. However, we believe that the NK cells down-regulate the development of effector cells, neutrophils and possibly also monocytes/macrophages, rather than exerting suppression during the inflammatory response itself.

INTERLEUKIN-2 INDUCES TYROSINE PHOSPHORYLATION OF STAT3- AND STAT5- LIKE PROTEINS IN HUMAN CD4 T CELL LINES

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Interleukin-2 is a critical regulator of T lymphocyte proliferation. The exact mechanism by which IL-2 receptors (IL-2R) regulate transcription of different genes is partly unknown. It was recently shown (1) that IL-2 induced tyrosine phosphorylation and nuclear translocation of STAT3, a newly identified member of the signal transducers and activators of transcription (STAT) family of proteins. Here we show, that IL-2 induces tyrosine phosphorylation of STAT3 and a STAT5- like protein in allospecific CD4 T cell lines. In contrast, STAT 1 and 2 proteins were not tyrosine phosphorylated. Interestingly, the STAT5- like protein had an apparent molecular mass of 100 kDa. STAT5 was originally identified as a 92 kDa protein in mammary epithelial cells (2) suggesting that the IL-2 responsive, 100 kDa protein may be a new, STAT5-like, member of the STAT family of proteins. Cytokine induced tyrosine phosphorylation of the STAT 3 and STAT5- like proteins followed similar kinetics. In conclusion, we provide new evidence that STAT proteins are involved in IL-2R signaling in antigen specific, human T lymphocytes.

1)Nielsen M et al Eur J Immunol 1994,24:3082. 2)Gouilleux F, et al 1994, 13-4361

NOICENTEVE MECHANISMS IN IMMUNOLOGICAL FUNCTIONING.

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A new hypothesis based on experimental work with various non-specific immunological stimulators is presented for better characterization and deeper understanding of the underlying processes in immunologic response.

Nocicepsis is the description of the disturbance of cell surface homeostasis by intimate physicochemical force leading to nerve cell stimulus. Nocicepsis can also be applied to the ignition phenomenon in the lympho-reticulo-endothelial defence mechanisms. The triggering force in the contact of intruding microbe,pollen or other non-self moiyet with the responding L-RES cell is excited by the electrochemical difference in the intimate cell surface milieu. This nonspecific electrochemical shock brought by the intruding agent leads then to revealing and activation of more specific signal mechanisms. This primary triggering is a well conserved unicellular phenomenon in contrast to the sequelae, where the functioning is dependent sophisticated cell to cell contacts and interactions.

Such evolutionally younger interaction mechanisms include various adhesion and immunological receptors as well the array of local and more general modifying mechanisms served by cytokines, inflammatory,allergic and neural mediators and various hormones. Their elementary function is to inhibit the spreading of the harmful element by needed amplification or suppression of various required cell poplations in a regulated manner leading in the end to extermination of the intruder.
T CELL EPITOPES OF THE MYCOBACTERIAL 18, 65, AND 70kD HEAT SHOCK PROTEINS: PEPTIDE DEFINED PRIMARY STRUCTURE AND HLA-RESTRICTION.

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M. tuberculos/M. leprae specific T cell lines and clones from healthy donors vaccinated with heat killed M. leprae or BCG were screened for proliferative reactivity against the recombinantly expressed heat shock proteins (HSPs) HSP18, HSP65, and HSP70. Overlapping synthetic peptides covering the respective HSP sequences were then used to map epitopes of the HSP reactive T cell lines or clones identified whereas a combination of blocking and panel studies were applied to identify the HLA molecules presenting the individual epitopes. Two epitopes of the HSP18 molecule presented by HLA-DR4, Dw4 and DR1, respectively, was identified. T cells recognized the mycobacterial HSP65 antigen in the context of HLA-DR1, DR3, DR5, DR7, and interestingly DR4 (Dw4 and Dw14). All of these epitopes except DR5 and DR7 recognition) were defined at the sequence level. By combining a DNA subclone and peptide approach, we identified T cell epitope sequences from HSP70 presented by HLA-DR2, DR3, and DR7. HLA-DR1, DR5, as well as HLA-DRw53 were shown to present epitopes not yet defined at the peptide level. The results will be discussed in relation to vaccine design.

The effects of serum concentration and factor I depletion on the deposition of C3 fragments on human B cells following spontaneous in vitro complement activation.

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In our attempt to optimize the deposition of C3 fragments on the surface of human B cells, we investigated the effect of increased serum concentration and depletion of factor I (FI) from normal human serum (NHS) on this deposition. We incubated peripheral blood leukocytes in 25-75% NHS from healthy AB-positive donors at 37°C. This was done in the presence of Mg-EGTA to block the classical complement pathway while allowing activation via the alternative pathway to continue. We then measured the deposition of C3 fragments on B lymphocytes, using FITC-conjugated polyclonal anti-C3c and C3d antibodies. The deposition was measured by flow cytometry, specifically selecting for B cells with PE-conjugated monoclonal anti-CD19 antibody. We found that increased serum concentration enhanced the deposition of C3 fragments on human B cells. Surprisingly the depletion of FI from NHS led to decreased deposition in spite of the fact that C3 conversion to C3 fragments in the fluid phase was enhanced. We conclude from this work that i) increasing the availability of complement enhances C3 fragment deposition on human B cells and that ii) this deposition is a result of local (surfacemembrane), rather than general (fluid phase) activation.

Phage selection of scFv antibodies binding to tissue expressed antigens.

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Recently we developed a method based on phage display technology for selection of scFv antibodies directed against novel antigens on the surface of viable cells. Now we present the development of a procedure for phage selection using tissue expressed antigens, i.e. the use of tissue sections to expose intracellular, membrane bound and extracellular tissue epitopes to phage antibody libraries from which binders of novel specificities can be selected. This would allow the discovery of new markers characterizing phenotypes expressed exclusively in vivo, such as pathological processes, cell-stromal interactions, organ-architecture dependent cellular phenotypes, markers of embryonic development or other markers of interest to histological in situ analysis. In model experiments phage reacting to an epithelial tissue antigen, C215, could be enriched 20-700 times over non-specific phage while pre-blocking with C215 Mab erased specific phage enrichment. Linear correlation of the enrichment factor versus the relative proportion of antigen positive surface area could be demonstrated. Finally, an immune library was subjected to positive selection to find scFv antibodies that could be demonstrated to bind to frozen sections of human tumor tissue.

CROSSLINKING OF THE T CELL-SPECIFIC ACCESSORY MOLECULES CD6 AND CD28 INDUCES T CELL ACTIVATION IN THE ABSENCE OF T CELL RECEPTOR/CD3 OCCUPANCY.

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The T cell surface molecules CD6 and CD28 have been shown to be receptors for accessory signals in T cell activation. We here demonstrate that in the absence of any other activating stimulus, simultaneous ligation of CD6 and CD28 by mAb induces T cell proliferation. An essential requirement for the induction of T cell proliferation via the ligation of both molecules is the immobilation of the anti-CD6 and anti-CD28 mAb by using rabbit anti-mouse immunoglobulins (RAM Ig). The T cell proliferation induced by crosslinking of CD6 and CD28 is IL-2 dependent, demonstrated by the cell surface expression of the IL-2R and the inhibition of the proliferative response by anti-IL-2 Mabs . In addition the proliferating T cells activated via CD6 and CD28 expressed IL-2 mRNA. The CD6/TPA-induced T cell proliferation was completely inhibited by cyclosporin A, however the proliferative response after crosslinking of CD6 and CD28 was partially inhibited by the immunosuppressive drug. Our data provide evidence for a pathway of antigen-independent T cell activation via CD6 and CD28, which is IL-2 dependent.
CLONING AND SEQUENCING OF cDNA ENCODING THE LEPIDOGLYPHUS DESTRUCTOR MAJOR ALLERGEN LEP d1

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Storage mites are a well recognized source for allergic diseases such as asthma and rhinitis. Lepidoglyphus destructor (Ld) is the most common storage mite species in northern and central Europe. An Ld population was obtained from Allergon AB, Angelholm, Sweden. Poly-A selected mRNA was partial cDNA cloning using standard methods. Degenerate primers were designed from the previously partially determined amino acid sequence of the major allergen of Ld, Lep d1. Reverse transcriptase polymerase chain reaction (RT-PCR) and PCR was performed using 3’RACE and 5’RACE protocols and the resultant cDNA fragments were cloned into TA plasmids (Invitrogen). The nucleotide sequences were computer analyzed using DNASIS software program. The probable leader sequence was 16 amino acid residues long. The encoding remainder of the sequence showed 125 amino acids residues for Lep d1. The full cDNA is approximately 550-600 nucleotides long. Comparison of several independent PCR products indicate polymorphism, the sequences show some altered amino acid composition in the encoding part and multiple mutations to the extent of block deletion in the non-coding part of the S’ and the 3’ end of the mRNA. The complete cDNA will be used in an expression system to obtain the recombinant allergen.

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IDENTIFICATION OF PNEUMOCOCCI BY THEIR PROTEIN ANTIGENS

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The common immunochemical identification and classification of Streptococcus pneumoniae is based on the properties of capsular polysaccharides. This approach does not cover noncapsular strains, which makes it difficult to study the role of these forms of bacteria, as well as forms with a small capsule, in human pathology. We analyzed variability of pneumococcal strains from the international collection by the isolation of immunochemically active pneumococcal proteins. The protein preparations of 46 pneumococcal strains of known serotypes were subjected to SDS gel electrophoresis with subsequent immunoblotting. Immunochemically active components were detected with the use of blood sera from patients with bacteriologically identified pneumococcal infection, as well as blood sera from clinically healthy adults. The presence of immunochemically active proteins with mol. wt. between 50 and 95 kD, as well as protein with a mol. wt. of ca. 80 kD, common for all strains under study, was established. Some of these proteins were common for strains of different serogroup types (strain 77/64 of type 22A and strain 39/57 of type 29), while some of them exhibited an identical pattern for one serogroup strains of one serogroup (strains 55/57 and 52/57, serogroup 35). The possibility of using this active protein antigens as markers of pneumococcal infection is considered. We could thus distinguish S.pneumoniae strains by some features other than the size of their capsules and their type. It would be interesting to find out the existence of epidemiological correlation between protein types and the morbidity rate and severity of pneumococcal infection.

ROLE OF CD4+ T-CELLS AND IgE IN MALARIA

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Malaria infections mean long lasting antigenic exposure of the human host. Such persistent stimulation affects the balance between CD4+ cells of Th1 and Th2 type with consequences for the course and final outcome of the infection. Th2 cell production of IL-4 is responsible for the switch of the immunoglobulin isotype production of B cells from IgM/IgG to IgE. Besides being the major immunoglobulin causing allergic reactions, IgE elevation is also a hallmark of helminthic infections. However high IgE levels have recently also been encountered in protozoal diseases, for example in Leishmania infections. More recently, we and others have also found significantly elevated levels of total as well as IgE anti-malarial antibodies in P.falciparum malaria. That IgE is induced by the malaria parasites is supported by the finding of elevated IgE in mice experimentally infected with P.chabaudi where the final clearance of the infection has been shown to be dependent on a Th2 response. In contrast we saw no elevation of IgE in mice infected with P.vinckei where immunity is dependent on a Th1 response. The influence of host genetic factors is suggested by the remarkable stability of the IgE antibody profiles over time and irrespective of season in an area with high and perennial transmission. Significantly higher IgE in Gambian children with cerebral P.falciparum disease than in uncomplicated cases might suggest that IgE is pathogenic in malaria. However IgE sensitized reactions involving cells equipped with Fcε receptors have been shown to efficiently kill helminthic parasites in vitro. Further studies will hopefully give insight in the question of IgE's role for protection and/or pathogenesis in malaria.
TARGETED DISRUPTION OF MURINE THIRD COMPLEMENT COMPONENT (C3) AND FACTOR B GENES IN VIVO

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Complement is a system of more than 30 proteins found both in plasma and on cell membranes. The complement system has several important functions in the immune response ranging from initiation of inflammation, neutralization and elimination of pathogens, and regulation of antibody responses, to clearance of immune complexes and disruption of cell membranes. Under certain conditions complement can, however, act as a mediator of deleterious inflammatory reactions and the complement activation has been implicated in the pathogenesis of some autoimmune disorders, bioincompatibility reactions and decompression sickness. Our objective is to disrupt in vivo mouse genes coding for the third complement component (C3) and factor B respectively. The C3-deficient mice will serve as a model of complement deficiency where the complement activation by any of the activation pathways is prevented at an early stage. These mice will be used to study the role of the complement system in the pathogenesis of some immune complex diseases, the regulation of antibody response, in the pathogenesis of decompression sickness and some bioincompatibility reactions. Factor B deficiency is expected to lead to selective absence of the initiation of inflammation, neutralization and elimination of pathogens, and regulation of antibody responses, to clearance of immune complexes and disruption of cell membranes. Under certain conditions complement can, however, act as a mediator of deleterious inflammatory reactions and the complement activation has been implicated in the pathogenesis of some autoimmune disorders, bioincompatibility reactions and decompression sickness. Our objective is to disrupt in vivo mouse genes coding for the third complement component (C3) and factor B respectively. The C3-deficient mice will serve as a model of complement deficiency where the complement activation by any of the activation pathways is prevented at an early stage. These mice will be used to study the role of the complement system in the pathogenesis of some immune complex diseases, the regulation of antibody response, in the pathogenesis of decompression sickness and some bioincompatibility reactions. Factor B deficiency is expected to lead to selective absence of the alternative pathway of complement activation and will be used to evaluate the role of this pathway of complement activation in the above described phenomena. The progress of the work will be presented.
and primed cytotoxic T cells (CTLs). In contrast to native Y
The bacterial superantigen Staphylococcus enterotoxin A (SEA) bind
directed against C215 transfected BI6 melanoma cells were seen
characterized by a reduced ability to produce IL-2. TNF-α and
amount of C215Fab-SEA injected i.v. induced high levels of
tumors in vivo, a recombinant C215Fab-SEA fusion protein reacting
particular T-cell receptor (TCR) Vβ chains. To target T cells to
MHC class II molecules and activates T lymphocytes expressing
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Human γ-globulin (HGG) was used as a model protein antigen for
covalet coupling to CTB and LTB respectively, and subsequent
comparision of these conjugates for oral-mucosal immunization or
tolariization respectively. Balb/c mice were perorally (p.o.)
immunized with the conjugates with or without cholera toxin (CT)
as extra immunomodulation. Locally produced antibodies were
measured by ELISA after mucosal tissue extraction of
immunoglobulins with a novel “PERFEXT” method. After feeding of
the conjugates, tolerance induction to parenteral antigen
injections was evaluated by DTH reaction. CTB-HGG conjugates
were superior to LTB-HGG conjugate for inducing local immunity
against HGG, whereas for induction of tolerance CTB-HGG and
LTB-HGG conjugates were equally efficacious. Addition of CT
strongly promoted local production of IgA, but abrogated, even in
submicrogram amounts, induction of tolerance to either type of
conjugate.

The bacterial superantigen Staphylococcus enterotoxin A (SEA) bind
to MHC class II molecules and activates T lymphocytes expressing
particular T-cell receptor (TCR) Vβ chains. To target T cells to
tumors in vivo, a recombinant C215Fab-SEA fusion protein reacting
with the human C215 colon carcinoma Ag, was constructed. The
immunopharmacology and tumor therapeutic effect of the C215Fab-
SEA fusion protein was investigated in C57Bl/6 mice. Microgram
amount of C215Fab-SEA injected i.v. induced high levels of
interleukin-2 (IL-2), tumor necrosis factor-α (TNF-α) and interferon-
γ (IFN-γ) and primed cytotoxic T cells (CTLs). In contrast to native
SEA no deletion of CD4+ cells expressing the SEA reactive TCR Vβ
3 or 11 was observed after repeated injections of C215Fab-SEA. On the
contrary, a 10 fold increase of CD4+ TCR Vβ 3+ cells was
observed. T cells responded to 3 consecutive injections with
C215Fab-SEA before they entered into a state of anergy,
characterized by a reduced ability to produce IL-2, TNF-α and IFN-γ
and failure to mediate cytotoxicity. A shift towards a Th2 pattern of
accumulation, and of NK cytotoxicity against YAC-1 cells.

We conclude that IFN-γ mediated resistance to T. cruzi is
associated to induction of NO release and increased expression of
functional receptors.

IMMUNOPHARMACOLOGY OF mAb TARGETED
SUPERANTIGENS
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ROLE OF INTERFERON-γ DURING MURINE INFECTION
WITH TRYPANOSOMA CRUZI.
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We studied the protective role of IFN-γ in the outcome of T,
cruzi infection, the causative agent of Chagas disease, by using
IFN-γ receptor knock out mice (IFN-γ R-). IFN-γ R- mice
showed dramatically enhanced parasitemia, tissue inflammation
in heart and skeletal muscle and cumulative mortality after infection
with a virulent (Tulahuén) or an avirulent (CA-I) strain of T.
cruzi. While a mononuclear cell infiltration was registered in the
heart and skeletal muscle from wild type mice 20 days after T.
cruzi inoculation, IFN-γR- mice displayed predominantly
polymorphonuclear cell infiltration. Spleen cells from T. cruzi
infected wild type mice showed increased expression of class I,
class II and B7.1 molecules, a phenomenon that was not
observed in IFN-γR- mutants. T. cruzi infection induces release of
nitrogen (NO) and oxygen reactive species. A partial inhibition in
H2O2 generation by spleen or peritoneal cells was registered in
IFN-γR- infected mice. NO release, as reflected by nitrate
levels in supernatants from cells infected from infected mice, nitrite
content in sera, or inducible NO synthase mRNA accumulation,
was completely abolished. IFN-γR- and wild type infected mice
displayed similar levels of IL-10, IL-4 and IL-6 mRNA
accumulation, and of NK cytotoxicity against YAC-1 cells.

We conclude that IFN-γ mediated resistance to T. cruzi is
associated to induction of NO release and increased expression of
functional receptors.

MONOCLONAL ANTIBODIES AGAINST SUBUNITS OF
CFA/I INHIBIT BINDING TO HUMAN ENTEROCYTES
AND PROTECT AGAINST CS4-EXPRESSING ETEC
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Enteroaggregative Escherichia coli colonize the intestine by means of
several antigenically distinct colonization factors (CFs). Antibodies
specific for whole CF fimbrae protect only against
bacteria expressing the homologous fimbrae. Several of the CFs
have considerable homology in amino acid sequence, e.g. in the
N-terminal end. We produced MAbs against CFA/I subunits that
cross-reacted immunologically with these CFs.

Methods: Isolated human enterocytes, prepared by scraping off
the mucosa from pieces of proximal intestine and using an EDTA-
chelation procedure, were mixed with a defined number of bacteria
expressing either CFA/I or CS4, which had been preincubated
with the different cross-reactive MAbs or a control
Bacterial adhesion was assessed by counting the number of bacteria adhering to the
brush borders of 150 enterocytes. Passive protection was done in
ligated rabbit small intestinal loops by incubating a mixture of
bacteria and MAbs and determining the fluid accumulation. The
binding of the MAbs to the fimbrae on whole bacteria was also
visualized with immunoelectromicroscopy.

Results: The different MAbs against CFA/I subunits inhibited
binding of both CFA/I- and CS4-expressing bacteria to human
enterocytes and also prevented fluid secretion caused by
homologous as well as heterologous strains. These results indicate
the possibility of inducing an immune response that can protect
against homologous as well as heterologous ETEC strains.
IMPAIRED RECOGNITION IN AUTOIMMUNE DISEASES: CLINICAL LABORATORY CONFIRMATION. V. Rosenkov, A. Belenkii, V. Krikunov, and B. Pinegin. 1 Kaunas Academic Clinics, Kaunas 3007, Lithuania, Fax: +370 7985855 2 Russian Medical Academy of Postgraduate Studies, and 3 Institute of Immunology, Moscow, Russia.

Autoimmune inclination is considered to be related with immune recognition defects. We studied the lymphocyte transformation response to mitogenic and allogeneic stimulation in 22 autoimmune patients suffering from Rheumatoid arthritis (RA), comparing with 15 healthy donors (HD) and 19 other patients suffering from Atopic bronchial asthma (ABA) and Aspirine asthma (AA).

The PHA and PWM stimulated lymphocyte blast transformation reaction (LBTR) in RA patients did not differ from HD, however the MLR response in RA was lower than in HD. So, while the allogeneic recognition stage precedes the proliferation stage, this reaction is diminished in autoimmune patients. In allergic persons the mitogenic response was stimulated, and the allogeneic reaction was increased correspondantly.

To demonstrate the accordance or disaccordance of LBTR and MLC in various pathologies we calculated the recognition/proliferation ratio (cpmMLR/cpmPHA or cpmMLR/cpmPWM). This ratio in HD was 0.74 ± 0.18 for PHA and 1.31 ± 0.54 for PWM. In ABA and AA group it was same: 0.65 ± 0.11 and 1.26 ± 0.21, while in RA recognition/proliferation ratio was evidently decreased: 0.47 ± 0.11 and 0.81 ± 0.10, accordingly, that shows the recognition ability failure.

So, in this way the recognition may be evaluated by the usual clinical immunologic tests.

ANTISYNTHETIC PEPTIDE POLyclonal ANTIBODIES REACTING WITH COLONIZATION FACTORS OF ETEC
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Enterotoxigenic Escherichia coli colonize the intestine by means of several antigenically distinct colonization factors. Several of these factors have considerable homology in amino acid sequence, e.g. in the N-terminal end. A CFA/I subunit MAb specific for the N-terminal end of CFA/I can inhibit attachment of both homologous and heterologous ETEC strains to enterocytes.

Methods: A peptide of the 25 N-terminal amino-acids of the CFA/I subunit protein was synthesized. Coupling to BSA was done by using the SPDP method. The accessibility of the peptide epitope on the conjugate was assessed by ELISA using the peptide-specific MAb. Rabbits were immunized four times s.c. with 500 μg of either uncoupled or coupled peptide using adjuvant. The sera tested in ELISA for reactivity with CFA/I peptide and several different purified CF fimbriae. Functional analyses were done by hemagglutination inhibition and by inhibition of binding to isolated human small intestinal enterocytes.

Results: Immunization not only with the conjugate but also with the unconjugated peptide resulted in equally high specific antibody titres against the CFA/I fimbriae and against the peptide. The titres against several other fimbriae were also increased but only against those with a related sequence. Interestingly, only antiserum against the coupled peptide could inhibit binding of ETEC bacteria to the cells. This suggests that it is possible to evoke a protective immune response against ETEC by using coupled peptides.

PRODUCTION of TNF-α, IL-1α/β and IL-10 by MUCOSAL MACROPHAGES IN INFLAMMATORY BOWEL DISEASE.
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Most macrophages in normal intestinal mucosa form a subepithelial band of cells with a mature phenotype (CD14+/L1+). In inflammatory bowel disease (IBD) macrophages with a monocyte-like phenotype (CD14+/L1+) occur. Production TNF-α, IL-1α/β and IL-10 was studied in these two subsets. Adherent mononuclear cells isolated from IBD lesions and normal mucosa were stimulated with IFN-γ/LPS or PWM. TNF-α and IL-1α/β production was examined by bioassays and paired immunofluorescence microscopy; production of IL-10, a potent downregulator of TNF-α in these cultures (unpublished data), was quantified by ELISA (Medgenics). IBD cultures depleted of CD14+ cells were compared with undepleted counterparts. In undepleted cultures TNF-α was significantly upregulated by PWM, IL-1 also after stimulation with IFN-γ/LPS which, however, did not significantly upregulate these cytokines in depleted cultures or normal controls. In PWM-stimulated IBD cultures, TNF-α and IL-1β appeared mainly in cells positive for the myelomonocytic antigen, L1. The production of IL-10 was increased in stimulated IBD cultures; upregulating the highest levels (x20 of unstimulated IBD cultures) after PWM stimulation; in these cultures depletion of CD14+ cells resulted in a significant reduction of IL-10 production (median 52%). Macrophages with a monocyte-like phenotype from IBD mucosa appear to be primed for production of TNF-α and IL-1, as well as for the macrophage deactivator IL-10. However, "physiological stimulation" (IFN-γ/LPS) upregulates only their IL-1 and IL-10 production, indicating a strong preference for downregulation of TNF-α production. Resident macrophages therefore do not appear to produce TNF-α as an early pathogenic step in IBD.

T CELL RECOGNITION OF P53 SUPPRESSOR GENE PRODUCTS BY HEALTHY DONORS AND CANCER PATIENTS.
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Mutational inactivation of the p53 tumor suppressor gene contributes to 50-60% of all human cancers. In normal cells only a few copies of the p53 protein can be detected. Mutations in the p53 gene increases the level of p53 expression in the cell and create new MHC I restricted epitopes for T cell recognition. Furthermore, the upregulation of wild type p53 epitopes might exceed the level of expression required for activation of autoreactive tumor specific T cells.

We selected peptides derived from the p53 sequence and tested their binding to HLA-A2.1 molecules using a biochemical assay. PBL from healthy HLA-A2.1+ donors were stimulated in vitro by autologous cells pulsed with p53 peptides. We detected T cell reactivity in these donors towards p53 epitopes with CTL precursor frequencies varying from 1:10^4 to 1:10^5. We isolated tumor infiltrating lymphocytes (TIL) from HLA-A2.1+ patients with colorectal tumors showing p53 mutations. These TIL's were expanded using high concentrations of IL-2 and immunobilized anti/CD3 mAb. The TIL lines were dominated by CD4+CD8+ cells and showed high TCR mediated cytotoxicity with little or no NK activity. In order to evaluate whether the TIL lines had reactivity against p53 peptides, we incubated the HLA-A2.1+ cell line T2 with a range of p53 peptides. p53 peptide pulsed T2 cells were specifically killed by some, but not all, of our expanded TIL lines.
The first T-cell receptor Jα-gene rearrangements
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We have analyzed TCR a-gene rearrangements during the early T-cell ontogeny by hybridoma production and PCR cloning. Our results of fetal thymocyte hybridomas demonstrate that the first TCR a-gene rearrangements are detectable on developmental day 16. Interestingly, in all fetal day 16 thymocyte hybridomas the most proximal Jα-gene (Ja50) was rearranged. This rearrangement was always found on one chromosome only with a concomitant δ rearrangement on the homologous chromosome. Four of the rearranged Ja50 genes were cloned and sequenced. In one hybridoma the V61D52-element, which is thought to be specific for the δ-region, was rearranged to Ja50-gene. It was not only found in hybridomas but it was detected in fetal thymus DNA by nested PCR as early as day 15.5 of development. Because the Ja50-gene is a pseudogene, which can not encode a functional TCR receptor on the cell surface, we wanted to determine whether thymocytes with this aberrant rearrangement could further develop. We used modified inverted-PCR technique to detect the replacement of the V61D52Ja50 rearrangement by secondary Ja -rearrangement. Thymus DNA from day 17.5 of development was amplified and the excision products which contained both the first Ja-region rearrangement (V61D52Ja50) and the reciprocal joint of the secondary Ja-region rearrangement (Va3Ja49) were sequenced.

We suggest that the V61D52Ja50-rearrangement belongs to the normal thymocyte development. Therefore our results also indicate that αβ and γδ lymphocytes have a common precursor.

ALTERNED MHC CLASS I PRESENTED PEPTIDE REPERTOIRE IS NOT SUFFICIENT TO INDUCE NK CELL MEDIATED F1-HYBRID RESISTANCE.
Margarita Salcedo, Petter Höglund, Adnane Achour, Christopher J. Thorpe and Hans-Gustaf Ljunggren. Microbiology and Tumor Biology Center, Karolinska Institutet, S-171 77 Stockholm, Sweden. Fax: +46 8 32 67 02.

Mature NK cells are known to mediate F1-hybrid anti-parental graft rejection responses. This phenomenon has been linked to the MHC, and in particular, to the α1/α2 domains of the MHC class I molecules. Here, we have addressed the role of MHC class I bound peptides in NK cell-mediated F1-hybrid anti-parental rejection by studying the resistance of F1-hybrids between B6 and different bm mutant strains to B6-derived RBL-5 lymphoma cell line. Tumor development occurred at a similar frequency in all combinations of (B6Xbm)F1 mice and control B6 mice. These results suggest that absence of a specific MHC class I presented peptide repertoire on grafted cells is not sufficient to induce NK cell-mediated F1-hybrid anti-parental rejection responses.

NATURAL KILLER CELL INTERACTION WITH MURINE ALLOGENEIC CLASS I MOLECULES.
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Class I molecules of the major histocompatibility complex (MHC) affect target cell sensitivity to natural killer (NK) cell lysis, and influence the development of the NK cell repertoire. In the mouse, absence of MHC class I molecules on target cells is associated with an increased susceptibility to NK cell mediated lysis, while syngeneic class I molecules confer protection. In contrast to the protective role of syngeneic class I molecules, less is known about the interaction between murine NK cells and allogeneic class I MHC molecules. In theory, such could either be triggering, inert or inhibitory. To directly address the role of allogeneic class I in interaction with NK cells of the mouse, a panel of polyclonal allogeneic murine NK cells were exposed to H-2b class I positive or negative target cells, and susceptibility to lysis was assessed. For all effectors studied, regardless of H-2 haplotype or genetic background, a preferential killing of class I deficient targets was observed. This pattern was observed in vitro with tumor as well as lymphoblast targets, and in vivo in rapid elimination studies of radiolabeled tumor cells. The results demonstrate that protection from murine NK cell mediated lysis can be conferred by the expression of allogeneic class I molecules. No evidence for a triggering effect caused by the expression of allogeneic class I molecules was observed. The data are discussed in relation to current models on NK cell/MHC class I interactions, alloseactivity mediated by NK cells, and the role of NK cells in allogeneic graft rejection responses.
CORTICOSTEROIDS AMELIORATE STAPHYLOCOCCUS AUREUS ARTHRITIS IN MICE
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Bacterial arthritis is a serious rapidly progressing disease with high morbidity and mortality despite antimicrobial therapy. The crostive arthritis is mediated by bacterial products as well as inflammatory cytokines. We evaluated the effect of corticosteroids in the treatment of septic Staphylococcus aureus arthritis.

Materials and methods. Swiss mice were injected i.v. with S. aureus LS-1. Three days after inoculation of bacteria i.p., with treatment with cloxazolin and dexamethasone was started. The mice were followed individually. Arthritis was evaluated clinically and histopathologically. Serum samples and bacteriological isolates were obtained.

Results. No treatment ClOXAZOLIN alone ClOXAZOLIN + dexamethasone Dexamethasone alone

| No. of mice | 27 | 27 | 29 | 13 |
| Mortality (%) | 22 | 11 | 7 | 15 |
| Occurrence of arthritis (%) | 81 | 48 | 22*** | 55 |
| S. aureus in serum (%) | 58 | 4* | 4* | 36 |
| Serum IL-6 | 1.47 +/- | 0.27 +/- | 0.81 +/- | 6.21 +/- |
| Serum NO3 | 49.8 +/- | 38.1 +/- | 29.6 +/- | 119.9 +/- |

Conclusions. Addition of corticosteroid therapy to the antimicrobial treatment of septic arthritis led to decreased mortality and arthritis. The outcome emphasizes the need of antimicrobial treatment along with antibiotic therapy to efficiently reduce the sequelae following S. aureus infection. Further studies using more selective immunosuppressants are going on.

HUMAN VASCULAR ADHESION PROTEIN-1 MEDIATES CARBOHYDRATE-DEPENDENT, SELECTIN-INDEPENDENT BINDING OF LYMPHOCYTES TO ENDOTHELIAL CELLS UNDER SHEAR STRESS
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The regulated interactions of leukocytes with vascular endothelial cells are crucial in controlling leukocyte trafficking between blood and tissues. Vascular adhesion protein-1 (VAP-1) is a novel, human endothelial cell molecule that mediates tissue-selective lymphocyte binding. Two species of VAP-1 exist in lymphoid tissues. Glycosidase digestions revealed that the mature 170 kD form of VAP-1 expressed on the lumenal surfaces of vessels is a heavily sialylated glycoprotein containing a mucin-like domain. The sialic acids were indispensable for the function of VAP-1, since the desialylated form of VAP-1 no longer mediated lymphocyte adherence. We also show that L-selectin is not required for lymphocyte binding to VAP-1 under conditions of shear stress. These data indicate that L-selectin negative lymphocytes can bind to peripheral lymph node type venules via VAP-1 mediated pathway thus circumventing the need for the MECA-79 defined peripheral lymph node addressin - L-selectin interaction. Moreover, our findings extend the role of carbohydrate mediated binding in lymphocyte-endothelial interactions beyond the selectins. In conclusion, VAP-1 naturally exists as a 170 kD sialomucin-like protein that uses sialic acid residues to interact with non-L-selectin ligand(s) of lymphocytes under non-static conditions.

Differences in GVHD reactions in SCID mice injected with T cells from CBA/J or C57BL/6 mice.
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The selection of allogeneic T cells after injection into neonatal SCID (H-2b) mice was analyzed. In the spleen of young recipients (≤ 21 days old) injected with CBA/J (H-2k) T cells, CD4+ donor cells had increased two times and CD8+ donor lymphocytes had increased six times in cell number, with a six-fold enhancement in the frequency of CD8+Vp14+ T cells. This was paralleled by clinical signs of GVHD disease. Donor cells from these mice did not proliferate in vivo when stimulated with spleen cells from nude mice of H-2b, H-2k, or H-2b haplotype. In eight weeks old recipient, however, spleens were of a size similar to that of untreated SCID, containing 25% donor lymphocytes with a normalised CD4/CD8 cell ratio and TCR Vβ repertoire, and the proliferative inhibition was confined to H-2k reactive donor cells. Thus, H-2k+ cells from these mice also proliferated against syngeneic non-T-cells, suggesting reversion of anergy of autoreactive populations when parked in SCID mice. When CBA/J donor cells that had resided in SCID mice for two months were transferred into syngeneic CBA/Ca nu/nu recipients a significant reversion from tolerance to H-2k expressing spleen cells was observed. When injecting C57BL/6 T cells, the SCID recipients developed a more severe GVHD resulting in death in the majority of the mice within three weeks. Here we also detected differences in the distribution of the donor cells and in the cytokine pattern in the sera compared to SCID mice injected with CBA/J. The significance of immunoregulatory cells in GVHD will be discussed.

T-cell responses to keratins and streptococcal M-proteins in psoriasis. H. Sigmundsdóttir, H. Valdimarsson, B. Sigurgeirsson*, M. Troye-Blomberg*, M.F. Good* and I. Jönsdóttir. Departments of Immunology and Dermatology, National University Hospital, Reykjavik, Iceland, *Department of Immunology, University of Stockholm, Sweden, and Molecular Immunology Unit, Queensland Institute of Medical Research, Brisbane, Australia. Fax: +354-5601943

Psoriasis is a chronic inflammatory disease characterized by hyperproliferation of keratinocytes and accumulation of activated T-cells in epidermis. Psoriasis has been associated with infections by Streptococcus pyogenes and cross-reactions between streptococcal M-proteins and human keratins have been demonstrated. The aim of this study was to investigate the possible role of cross-reactive T-cells in the pathogenesis of psoriasis. Peripheral T-cell responses to keratin, M-protein and four M-peptides with shared sequences were analysed in untreated psoriatic patients and healthy controls by proliferation and production of IFNγ and IL-4 using ELISPOT. There was a dominating IFNγ response to M-proteins and keratin, characteristic for Th1-cells, with little IL-4 production and proliferation. Compared to controls psoriatic patients showed markedly stronger IFNγ-response to all the peptides, but less difference was observed in response to whole proteins. These results are consistent with the hypothesis that streptococcal infections may activate keratin reactive Th1-cells in susceptible individuals, contributing to the pathogenesis of psoriasis.
T-CELL RESPONSES TO KERATINS AND STREPTOCOCCAL M-PROTEINS IN PSORIASIS. H. Sigmundsdóttir, H. Valdimarsson, B. Sigurgeirsson, M. Troye-Blomberg, M.F. Good and I. Jónsdóttir. Departments of Immunology and Dermatology, National University Hospital, Reykjavik, Iceland, *Department of Immunology, University of Stockholm, Sweden, and *Molecular Immunology Unit, Queensland Institute of Medical Research, Brisbane, Australia. Fax: +61-7-354-5601943

Psoriasis is a chronic inflammatory disease characterized by hyperproliferation of keratinocytes and accumulation of activated T-cells in epidermis. Psoriasis has been associated with infections by *Streptococcus pyogenes* and cross-reactions between streptococcal M-proteins and human keratins have been demonstrated. The aim of this study was to investigate the possible role of cross-reactive T-cells in the pathogenesis of psoriasis. Peripheral T-cell responses to keratin, M-protein and four M-peptides with shared sequences were analysed in untreated psoriatic patients and healthy controls by proliferation and production of IFNγ and IL-4, using ELISPOT. There was a dominating IFNγ response to M-protein and keratin, characteristic for Th1-cells, with little IL-4 production and proliferation. Compared to controls psoriatic patients showed markedly stronger IFNγ-response to all the peptides, but less difference was observed in response to whole proteins. These results are consistent with the hypothesis that streptococcal infections may activate keratin reactive Th1-cells in susceptible individuals, contributing to the pathogenesis of psoriasis.

RELEASE OF SULFIDOLEUKOTRIENES IN VITRO - RELATION TO ROUTINE SKIN TESTS IN THE DIAGNOSIS OF ALLERGY

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Skin testing was compared with in vitro production of sulfidoleukotrienes (sLT) in the diagnosis of immediate hypersensitivity, because a reliable in vitro test offers obvious clinical and financial advantages over an in vivo test. Patients. Eight children with asthmatic symptoms and one adult with atopic eczema were tested. Allergens were the most common ones in the living environment of the patients: dog, cat, horse, timothy grass, birch, fish, egg and milk. Skin tests were performed with routine prick testing. sLT were released from isolated leucocytes in the presence of allergen and measured by a novel test (Bühlmann Laboratories, Basel, Switzerland), the Cellular Allergen Stimulation Test (CAST-ELISA) based on the detection of LTC4, LTD4 and LTE4 by a monoclonal antibody. Correlation between skin test and sLT production:

| sLT production | Skin test + | Skin test - |
|----------------|------------|------------|
| +              | 22         | 4          |
| -              | 3          | 37         |

The concordance was 89%. Conclusion: sLT is suitable in routine diagnosis of immediate allergy.

MHC CLASS I SIGNALING IN HUMAN T-CELLS

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Cross-linking of MHC Class I molecules (MHC-I) induces signals in human T-cells. We have studied the biochemical signal-pathway that leads to IL2 production and IL2 receptor (CD25) upregulation. Our results suggest that MHC-I signaling is initially dependent on tyrosine kinase activity which induces tyrosine phosphorylation and activation of PLC-γ1, leading to a rise in the intracellular free Ca2+ concentration. MHC-I signal transduction is dependent on surface expression of the TCR/CD3 and CD45 molecules. We are presently studying whether any specific tyrosine kinases are activated following MHC-I cross-linking.

We have also investigated the later distal signal-pathway. Preliminary results suggest that an hereto unknown protein is specifically translocated to the cell nucleus following MHC-I cross-linking. The nature of this protein is currently under investigation.

THE COURSE OF DISEASE IN *PLASMODIUM BERGHEI* MALARIA-INFECTED C57BL/6 MICE DEPENDS ON THE SIZE OF THE INFECTION INOCULUM.

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Infection of inbred C57BL/6 mice by asexual blood-stages of *P. berghei* malaria parasites invariably results in a fatal disease course. However, there are two distinct clinical syndromes, related to the size of the infective inoculum: Either death occurs during the second week of infection with marked neurological symptoms (known as experimental cerebral malaria or ECM), or several weeks later as the result of gross anaemia and hyperparasitaemia without any apparent neurological involvement. We have investigated in detail how the course of infection in this system depends on the size of the infective inoculum. Mice were infected intraperitoneally with 1×10² to 1×10⁶ blood-stage *P. berghei* K173 parasites, and the course of infection was followed daily by measuring parasitaemia, reticulocyte count, and rectal temperature.

Low infection doses resulted in ECM in all mice, while no mice developed ECM after high dose infection. Intermediate dose infection caused some but not all mice to develop ECM. Detailed results of these experiments will be presented, and the possible background for the dependency of the clinical outcome on the size of the infection inoculum will be discussed.
INTERFERON ALPHA SECRETING CELLS IN HEMATOPOIETIC ORGANS OF PIG FETUSES AFTER IN VIVO STIMULATION BY CORONAVIRUS TGEV

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Coronavirus transmissible gastroenteritis virus (TGEV) induces an acute and fatal diarrhea in newborn piglets, as well as an intense and early production of interferon α (IFN-α). We have previously shown that in vitro inducible porcine IFN-α secreting cells (IFN-α SC) were present in pig fetal hematopoietic organs at early stages of gestation (Špíchal et al., Immunol. Letters, 1994, 43, 203-208). In the present study, miniature pig fetuses were treated by TGEV via umbilical cord vein injection after laparotomy and uterotomy of anaesthetized pregnant sows. Control fetuses were subjected to the same surgery. All fetuses were delivered 20 hrs after surgery. Cell suspensions from blood, liver, bone-marrow and spleen were incubated for 10 hrs in tissue culture microplates or nitrocellulose-bottomed microplates coated with anti-pig IFN-α mAb. IFN-α titers in supernatants were determined by a specific ELISA, whereas IFN-α SC frequency was obtained by an ELISPOT assay. Flow cytometry was performed to characterize CD 45, CD 4, SLA class II and 74-22-15 G/M positive cells. IFN-α SC and IFN-α production were detected after in utero TGEV injection of fetuses at the second half period of gestation. IFN-α SC were more abundant in non-adherent cell preparations than in total cell suspensions. These results show the presence of IFN-α SC in porcine fetal lymphohematopoietic organs following in utero exposure to TGEV.

ANTIBODY RESPONSE IN WASTE COLLECTORS AFTER MICROBIAL EXPOSURE DURING WASTE COLLECTION.

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Waste collectors are exposed to a variety of different microorganisms during collection of waste. These microorganisms are mainly fungi and actinomycetes, and to a lesser extent bacteria. Investigations were performed with waste collectors collecting compostable waste in order to determine the microbial exposure and their specific antibody response against microorganisms. Waste collectors were equipped with filters for collection of airborne microorganisms. The microorganisms found on filters were cultivated on selective media. The predominant microorganisms were found to be fungi of the Penicillium and Aspergillus species, and actinomycetes. Serum from the waste collectors was investigated for antibody response (IgG, IgE, IgA) against the cultivated microorganisms in SDS-PAGE and immunoblotting. Various antibody responses (IgG) were found against Aspergillus fumigatus, which is a fungus that can give respiratory symptoms. Other antibody responses were found against different microorganisms cultivated from exposed filters. These are being further investigated. This study is a part of the research programme "Waste Collection and Recycling" (CORE).

METHOD EVALUATION OF AN ENZYME-LINKED IMMUNOSORBENT ASSAY FOR DETERMINATION OF IgA IN SERUM.

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Comparability of measurement results is essential for research studies. Validation of analytical methods by a method evaluation study is therefore required before methods are used for research purposes. In this study a method evaluation of an Enzyme-Linked Immunosorbent Assay (ELISA) for determination of IgA in human serum was performed by use of the programme AMIQAS. The ELISA was set up as a sandwich ELISA using antibody against IgA for coating microtiterplates. Method evaluation samples were prepared from a serum sample with a high concentration of IgA (6.5 g/L) and an IgA-deficient serum sample in the ratio 1+5, 2+4, 3+3, 4+2, 5+1, 6+0. The serum samples were run in a dilution of 1/40,000. The detection range established by this method evaluation was shown to be 0.3 - 6.5 g/L.

The method evaluation analysis showed that the method was in analytical and statistical control. No significant systematical errors could be demonstrated.

IgA may be used as a marker of exposure to microorganisms or to stress. The method is currently being used for investigations of IgA levels in serum from waste collectors and from workers employed in the textile industry.

MANNAN-BINDING PROTEIN FORMS COMPLEXES WITH ALPHA-2-MACRoglobulin. A PROPOSED MODEL FOR THE INTERACTION.

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We report that alpha-2-macroglobulin (α2M) can form complexes with a high molecular weight porcine mannin-binding protein (pMBP-28). The α2M/pMBP-28 complexes were isolated by PEG precipitation and affinity chromatography on mannan-Sepharose, protein A-Sepharose and anti-IgM Sepharose. The occurrence of α2M/pMBP-28 complexes was further indicated by use of an anti-α2M affinity column and chelating Sepharose loaded with Zn²⁺. The eluates from these affinity columns showed α2M and pMBP-28 in SDS-PAGE and Western blotting. Furthermore, the α2M/pMBP-28 complexes were demonstrated by electron microscopy. Fractionation of pMBP-containing D-mannose eluate from mannan-Sepharose on Superose 6 showed two protein peaks which reacted with anti-C1s antibodies in ELISA, one of about 750 kDa which in addition contained pMBP-28 and anti-α2M reactive material, the other with an M, of 100-150 kDa. The latter peak revealed rhomboid molecules (7 x 15 nm) in the electron microscope and a 67 kDa band in SDS-PAGE under reducing conditions. This band was also seen in eluates from the anti-α2M and chelating columns. Based on these observations we propose a model for the interaction of pMBP-28 with α2M.
Molecular Mechanisms of Superantigen Induced T Cell Anergy in Vivo

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Anergy is a major mechanism to ensure antigen-specific tolerance in mature T lymphocytes. In vivo, anergy has mainly been defined at the cellular level. We utilized the T-cell activating superantigen staphylococcal enterotoxin A (SEA) to investigate the molecular mechanisms of T lymphocyte anergy in vivo. Injection of SEA to adult mice activated CD4+ T cells expressing certain T-cell receptor (TCR) Vβ-chain families, and induced a strong and rapid production of interleukin-2 (IL-2). In contrast, repeated injections of SEA caused CD4+ T cell deletion and anergy in the remaining CD4+ T cells, characterized by reduced expression of IL-2 at mRNA and protein levels. The failure to produce IL-2 was further examined at the transcriptional level. We analyzed expression of the transcription factors AP-1, NF-κB and octamer binding proteins, which are known to be involved in the regulation of the IL-2 gene promoter activity. Distinct different patterns of AP-1 and NF-κB transcription factors were found in anergized and activated CD4+ T cells.

SIMULTANEOUS ANALYSIS OF INTRACELLULAR INTERFERON-α AND CELL SURFACE ANTIGENS ON NATURAL INTERFERON-α PRODUCING CELLS BY FLOW CYTOMETRY.

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The interferon-α (IFN-α) producing blood leukocytes, stimulated by Herpes simplex virus (HSV), lack markers of B- and T-cells as well as of monocytes and NK-cells. This so called natural IFN-α producing cells (NIPCs) do express CD4 and HLA-DR antigens, and may belong to the null-cell population. To further clarify the identity of NIPCs, and in particular their relationship to dendritic cells (DCs), a double labelling technique for simultaneous detection of intracellular IFN-α and expression of cell surface antigens was established.

Human peripheral blood mononuclear cells (PBMCs) were stimulated with HSV to produce IFN-α, fixed in paraformaldehyde and permeabilized. The cells were then stained with monoclonal antibodies (mAbs) reactive with various cell surface antigens and with mAbs to IFN-α, and subsequently analyzed by flow cytometry (FCM).

Previous phenotypic characterizations of HSV-stimulated NIPCs were confirmed, and in addition the NIPCs were found to be positive for the CD40, CD44 and CD45RA antigens, but negative for FcεRI and B7-2.

Further, the ability of NIPCs to stimulate antigen-specific T-cell responses was tested. The PBMCs were stimulated with HSV, fixed and stained for intracellular IFN-α. The NIPCs were sorted with FCM, and shown to stimulate the proliferation of autologous T-cells from HSV-immune, but not non-immune, donors.

In conclusion, NIPCs resemble but are not identical to DCs, and have the ability to present antigens to immune T-cells.

Guillain-Barré’s syndrome. Possible effect of subgrouping on effect of plasma-exchange.

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The GB-syndrome has been recognised not always to have a favorable course, plasma-exchange (PE) or high-dose immunoglobulin treatment, although influencing beneficially the early course, seems to influence the long-term prognosis in a much less certain way.

The individual course of the disease is very difficult to predict, the early course giving some clues to later deficits of motor function. Previous gastrointestinal infection by Campylobacter jejuni species has been associated directly with pathogenic, immuno-inflammatory mechanisms of the disease, but is still only among highly varying events of both infections and unspecific episodes.

93 consecutively admitted patients fulfilling criteria for GBS have been retrospectively evaluated. 64 pts. did have severe course indicating PE. The 25 pts. served as historical controls, 39 pt had PE performed. Subgrouping according to episode 1-3 weeks before paralysis started seemed to influence outcome of treatment. Speculations about possible mechanisms are presented.

Early Intrathecal Production of Interleukin-6 Predicts the Size of Brain Lesion in Stroke

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The aim of the present study was to investigate patterns of local inflammatory responses as a consequence of acute stroke.

Methods: Thirty stroke patients were studied prospectively on days 0-3, 7, 21, and 90 with clinical evaluations, radiological assessments and analysis of serum and cerebrospinal fluid (CSF) cytokine levels.

Results: Significantly increased levels of IL-6 in CSF (p≤0.001) were observed in virtually all the stroke patients studied compared to healthy controls. This increase was observed during the whole observation time but was significantly more pronounced within the first days after the stroke onset with a peak level on days 2 and 3. This initial increase was significantly correlated (R=0.65; p=0.002) with the volume of infarct measured by MR 1-2 months later. Serum levels of IL-6 were significantly lower those of CSF (p=0.013) and did not display any significant correlation to the size of the brain lesion. Also, increase of intrathecal but not systemic IL-1β levels was observed early during the stroke.

Conclusion: An intrathecal production of IL-6 and IL-1β in patients with stroke is demonstrated, supporting the notion of localized inflammatory response to the acute brain lesion. The significant correlation between early intrathecal production of IL-6 and the subsequent size of the brain lesion can be used as a prognostic tool, predicting the size of the brain damage before it is possible to accurately visualize it with radiological methods.
NATIVE HUMAN SERUM AMYLOID P COMPONENT IS A SINGLE PENTAMER. I. Juul Sørensen, O. Andersen, E. Holm Nielsen and S-E. Svehag. Depts. of Medical Microbiology and Anatomy and Cytology, Odense University and Dept. of Infectious Diseases, Odense University Hospital, DK-5000 Odense C, Denmark. Fax +45 65 915267.

Serum amyloid P component (SAP) and C-reactive protein (CRP) are members of the pentraxin protein family. SAP is the precursor protein to amyloid P component present in all forms of amyloidosis. The prevailing notion is that SAP in circulation has the form of a double pentameric molecule (decamer) whereas CRP is a single pentameric molecule.

We have investigated by gel permeation chromatography the M, of SAP in freshly collected human serum and of SAP purified by carbohydrate affinity chromatography and anion exchange chromatography. SAP was monitored by quantitative immunoelectrophoresis and ELISA, and SAP peak fractions were analyzed by use of SDS-PAGE, Western blotting, and electron microscopy. The results indicate that native SAP circulates as a single pentamer, part of which forms complexes with C4b-binding protein. The properties of SAP changed during purification as indicated by immunoelectrophoresis and electron microscopy. Thus, electron micrographs of purified SAP showed a predominance of decamers. However, the SAP decamers reversed to single pentamers when purified SAP was incorporated into SAP-depleted serum.

COMPARISON OF RHEUMATOID FACTORS PRODUCED IN HEALTH AND RHEUMATOID ARTHRITIS. K.M. Thompson, M. Berretnsen, I. Randen, Ø. Ferre, J.B. Natvig, Institute of Immunology and Rheumatology, The National Hospital, Fr.Qvams gt.1, 0172 Oslo, Norway.

We have compared the structure of IgM rheumatoid factors (RF) produced as monoclonal antibodies from healthy individuals, following immunization, to RF derived from RA patients. RF from the synovial tissues of RA patients preferentially use Va 3 gene segments together with a diverse array of Va. This contrasts with RF derived from the peripheral blood of healthy donors, where the great majority utilize light chain segments of the kappa 3 family, and Va 1 heavy chain segments are frequently used. RF from both groups were frequently found to be extensively mutated in the Va segment. However, inspection of the R:S ratios in the CDR1+2 of these antibodies revealed that there was a strong selective pressure acting against mutations leading to amino acid replacements in the RF from healthy individuals. This was not seen in RF from RA patients. Affinity measurements indicate that RF in healthy individuals do not undergo affinity maturation, and that RF in RA may develop high affinity for IgG which may be of significance to the pathology of the disease.

ESTROGEN REDUCES LYMPHOCYTE HOMING TO RHEUMATOID SYNOVIM, GRAFTED INTO SCID MICE.

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Aim: To study estrogenic effects on lymphocyte migration to inflamed rheumatoid synovium.

Method: Immunodeficient SCID mice were subcutaneously engrafted with small pieces of synovial tissue from patients with rheumatoid arthritis (RA). Recipient mice were treated with various doses of 17β-oestradiol, dexametason or cyclofosamid. Three weeks later mice were injected i.p. with 10^7 freshly isolated ^3Cr labelled human lymphocytes. After 48 hours mice were sacrificed and the synovial graft as well as salivary glands were removed. The homing of labelled lymphocytes to synovial graft was evaluated in a y-counter and expressed as cpm/mg tissue. Cell migration on single cell level was confirmed using autoradiographic technique on frozen tissue sections.

Results: Synovial grafts in SCID mice treated with 17β-oestradiol displayed significantly (p<0.05) lower number of infiltrated human lymphocytes compared with controls. In contrast, no reduction of lymphocyte migration was observed in synovial grafts of mice treated with cortisol or cyclofosamid. None of the treatment regimens altered lymphocyte migration to murine salivary glands.

Conclusion: Our study shows that estrogen has the capacity to selectively reduce the influx of lymphocytes into the inflamed human synovium. This finding could be of relevance for the understanding of the ameliorating effect of estrogen in human RA patients. Studies of the mechanism(s) by which estrogen inhibits lymphocyte homing is ongoing.

IMMUNE RESPONSES AGAINST HOMOLOGOUS AND HETEROLOGOUS HELICOBACTER PYLORI ANTIGENS

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Preliminary studies suggest the existence of several different serotypes of H. pylori. In this study, the capacity of H. pylori to induce type-specific immune responses was studied. Sera and gastric aspirates from 11 H. pylori infected Swedish adults were tested for specific antibodies against membrane preparations (MPs) and lipopolysaccharides (LPS) prepared from the study subjects' own strains and with corresponding antigens from two reference H. pylori strains using different ELISA methods. It was found that sera from 6 of the 11 subjects had significantly higher IgA antibody titers and 4 sera had higher IgG titers against MPs from the homologous strains than from the reference strains. Five sera had higher IgA titers and 5 sera had higher IgG titers against LPS prepared from the subject's own strain than against LPS preparations from the two reference strains. Similarly, gastric aspirates from six subjects had higher IgA titers against the homologous than the heterologous MPs and five aspirates reacted in higher IgA titer with the homologous LPS. In immunoblotting analyses, sera showed stronger reactivities with homologous than with heterologous LPS preparations.

The results support the existence of different serotypes of H. pylori expressing strain-specific antigenic determinants that are immunogenic in vivo.
Mice of various inbred strains of conventional euthymic mice were fed by gliadin in attempts to induce enteropathy, Gliadin was administered intragastrically for two months starting in 10 days old mice, bovine serum albumin (BSA) fed mice served as controls. As conventional mice were not sensitive to gliadin feeding, immunodeficient strains of mice: athymic nu/nu Balb/c mice, mice with targeted disruption of IL-2 gene (IL-2 K.O.) and mouse strain with severe combined immunodeficiency (SCID), were used to determine the possible effect of long term gliadin feeding on their intestinal mucosa. Profound changes of intestinal mucosa were found in specimens of jejuna of gliadin fed athymic nude mice. Computer image analysis revealed markedly lower values of villi lengths and areas in gliadin fed nude mice as compared with BSA fed controls. IL-2 K.O. and SCID mice exert only minimal changes. The differences in response to gliadin feeding among various experimental models suggest that impairment of immunoregulatory mechanism is responsible for induction of intestinal lesions.

**Cytokine Production by Peripheral Blood and Synovial Tissue Cells in Rheumatoid Arthritis (RA)**

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T lymphocytes accumulate in the rheumatoid synovium. The T cell derived cytokines IFNγ and IL-4 are produced following activation and affect the activity of other cells in the compartment. However, there are conflicting results concerning the production capacity of cytokines in the synovium. In this study we compared the in vitro activation of T-lymphocytes from synovial tissue (ST) and peripheral blood (PB) in patients with RA by measuring IFNγ and IL-4 levels after in vitro stimulation of mononuclear cell suspensions.

**Methods:** ST and PB samples from 18 patients with RA were obtained. Mononuclear cell suspensions were cultured for 72 hours in medium containing ConA. Cell-free supernatants were analysed for IFNγ and IL-4 contents.

**Results:** No spontaneous production of IFNγ was detected in any of the PB or ST cultures. In contrast, following stimulation with ConA was detected in 12/14 PB (3400+42000/ml) and 13/14 ST (3300+6300/ml) cultures. A spontaneous IL-4 production was detected in 8/17 PB (600+300pg/ml) and 8/17 ST (100+400pg/ml) cultures. A mitogen-induced IL-4 production was detected in 12/16 PB (3200+4100pg/ml) and 13/16 ST (900+1900 pg/ml) cultures. In 10 of these 16 patients the synovial IL-4 production was lower than in matched PB samples.

**Conclusion:** No significant differences in the activation between PB and ST T-lymphocytes are found with respect to the mitogen induced synthesis of IFNγ and IL-4. In addition, there was no apparent dominance of Th1 or Th2 population in any of the two compartments analysed in patients with RA.

**Idiotyp e1 to Haemophilus influenzae Type b, Induced by Cross-Reactive Antigens**

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The Chinese population in Hong Kong has extremely low incidence of invasive H. influenzae type b (Hib) infection as well as of carriage of the microorganism. Although there is no vaccination against Hib in Hong Kong significant amount of IgG antibody against Hib capsular polysaccharide (CP) was found in healthy children and adults with ELISA (Lau et al.,1994). The origin of these antibodies may be cross-reactive antigens like CP of E.coli K 100 which is very similar to Hib CP. The aim was to determine the isotype and idiotype distribution of natural antibody against Hib CP in healthy Hong Kong residents. ELISA of 20 sera using biotinylated Hib CP showed antibodies against Hib CP in all individuals. The antibodies were mainly IgG2, but in most samples also IgA and IgG4. Three individuals had IgG1 and two IgM antibodies. The only child had the highest IgG1 antibody level. Isoelectric focusing/immunoblotting (IEF) patterns of IgG2 antibody clones against CP of Hib and E.coli K 100 were very similar in almost all cases. Three individuals had additional clones for K 100 CP as compared to Hib CP, which disappeared after absorption with K 100 CP in two cases. Spectrotyptic analysis of IgG antibodies belonging to the specific IgG2(IgG2a-IgG2d) Clusters (Lucas et al.,1991) revealed much stronger IEF patterns as compared to the Hib CP specific IgG2 antibody with bands in different locations. The high reactivity of serum IgG, IgA and IgM against mononuclear antibody to Hib Id-1 (a-Id-1) was also found in ELISA. This reactivity was not abolished after absorption of the sera either with Hib CP or K 100 CP. The data indicate the high prevalence of Id-1 in the Hong Kong population. However, the share of Id-1 among Hib CP specific antibodies was significant only in one individual. Others had much lower activity of Id-1 anti-Hib CP antibodies as compared to the total IgG Id-1. That may indicate that the Hong Kong subjects have Id-1 positive antibodies in their serum that are not specific for Hib CP. This is consistent with the nature of Id-1 which is a marker of the A2V1 region usage rather than a marker of Hib CP paratopes. We suggest that natural antibodies to Hib CP found at high levels in the sera of healthy Hong Kong Chinese are the product of exposure to cross-reactive antigens different from E.coli K 100 CP.
BONE MARROW T CELL PROGENITORS EXPRESS A NOVEL MOLECULE OF THE IG SUPERFAMILY.

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Colonization of the embryonic thymus by hematopoietic progenitors depends on the adhesion of these cells to the thymic vascular surface, extravasation, and transepithelial migration. Using the chicken, which offers excellent access to the embryo, we searched for T cell progenitor-specific antibodies that could interfere with thymus homing. Monoclonal antibody (mAb) 2-264 recognized thymocytes from embryonic day 12 onwards, but the expression level of the antigen decreased upon thymocyte differentiation. In the adult, only low expression was found on 5-10% of blood and spleen cells. The expression of this protein is highest on CD4^+8^- thymocytes, intermediate on CD4^+8^- thymocytes, and low on CD4^-8^- thymocytes. CD4^-8^- thymocytes lack the 2-264 antigen. A subpopulation of embryonic bone marrow cells and vascular endothelial cells are 2-264+. From thymocytes and a transformed T cell line, mAb 2-264 precipitated a molecule of 110 kD both under reducing and nonreducing conditions. An embryonic chicken thymocyte cDNA library in the vector pcDNA3 was screened in a COS cell transient transfection system, and a 1.8 kb cDNA clone was isolated. The protein predicted from the nucleotide sequence is an adhesion molecule belonging to the immunoglobulin superfamily. It consists of five extracellular Ig domains, a transmembrane region, and a short cytoplasmic tail. As 2-264+ cells in 13-day embryonic bone marrow in a high frequency give rise to T cells after intrathymic reconstitution in the congenic chickens, we suggest that this protein is involved in T cell precursor migration into the thymus.

MECHANISMS RESPONSIBLE FOR THE IMPAIRED MUCOSAL IMMUNE RESPONSE IN IL-4 KNOCKOUT MICE

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We have found that IL-4-targeted mice (IL-4/-) are grossly impaired in their mucosal immune response to conventional protein antigens. In the present study we sought to understand the mechanisms responsible for the defect in these mice. Wild type and IL-4 +/- mice were orally immunized with OVA together with cholera toxin (CT) adjuvant. Irrespective of the dose of antigen we failed to detect but poor anti-OVA local and systemic responses while anti-CT responses were roughly 50% of those found in wild type mice. Even when IL-4 +/- mice had already responded with high anti-OVA serum antibody titers after i.v. immunization we could not elicit local anti-OVA IgA responses through oral immunizations. Further attempts to immunize perorally with OVA entrapped in microparticles failed. By contrast, if OVA was covalently coupled to CT strong responses were detected both systematically and in particular in gut lamina propria. Whereas only poor T cell priming in gut associated tissues was achieved with OVA given admixed with CT the conjugated form of OVA fully restored specific T cell priming as determined by proliferation and IFN-y production. Contrary to what has been proposed earlier CT acts in the absence of IL-4, and probably Th2-cells, and uses an IL-4 independent pathway to stimulate gut mucosal immune responses.

PARTICIPATION OF T LYMPHOCYTES IN HEMATOGENOUSLY ACQUIRED S. AUREUS NEPHRITIS

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Staphylococcus aureus is the most prevalent infectious agent in hematogenously spread kidney infections in man. The aim of our study was to develop a model of this disease and to characterize in situ inflammatory reactions.

Materials and methods: Sixty Swiss mice were inoculated i.v. with 0.8 x 10⁷ S. aureus of LS-1 strain. At days 1, 4, 7, 14, 21 and 32 after inoculation the mice were sacrificed. Bacteriologic cultures from blood, urine and kidneys were obtained. In addition, histopathological and immunohistochemical evaluation of kidneys was performed.

Results: Within the first week after inoculation peak values of tissue resident S. aureus were observed. Histopathologically both focal and diffuse inflammatory infiltrates were seen in kidney cortex and medulla. Immunohistochemical evaluation revealed high numbers of Mac-1^+ phagocytic cells as well as CD4^+CD8^+ lymphocytes. Many T cells stained for V beta 4, 7, 10b and 11 TCR families.

Conclusions: We describe a first model of hematogenously acquired S. aureus nephritis. Immunohistochemical data suggest that T lymphocytes vividly participate in the course of this disease.

Role of ICAM-1 in Staphylococcus aureus septicemia and arthritis

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Intercellular adhesion molecule 1 (ICAM-1) is a member of the immunoglobulin superfamily interacting with the integrins. This interaction is critical for leukocyte extravasation into inflamed tissue. The aim of this study was to assess the role of ICAM-1 in the pathogenesis of Staphylococcus aureus septicemia and arthritis.

Method: Mutant mice, lacking the ICAM-1 gene, and normal wild-type congenes were intravenously injected with S. aureus strain LS-1. The animals were followed-up clinically during six days, regarding development of arthritis and mortality.

Results: Six days after inoculation 50% of the animals in the ICAM-1 mutant group have died but none in the wild-type group. The mutants developed less severe arthritis than the wild-type animals. Histopathological examination of the ICAM-1 deficient mice displayed decreased erosive arthritis than in the controls. The sera from the mutants contained lower levels of IFN-γ compared to the congenes. This is in agreement with the in vitro data, where spleen cells from naïve ICAM-1 deficient mice stimulated with TSST-1 and cell walls, produced lower levels of IFN-γ than spleen cells from healthy controls. Naïve mutant mice had more circulating neutrophils and lymphocytes but lower number of cells capable of phagocytosis than the wild congenes.

Conclusion: Mice lacking the ICAM-1 gene exhibit higher mortality in S. aureus arthritis than normal controls. Lower levels of IFN-γ production and lower number of phagocytizing cells compared to the wild-type mice may contribute to this. However, decreased extravasation of leukocytes due to the ICAM-1 deficiency leads to a less destructive course of septic arthritis in comparison with the wild littermates.

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T CELL RECEPTOR V GENE USAGE BY LUNG AND PERIPHERAL BLOOD T LYMPHOCYTES IN PATIENTS WITH EXTRINSIC ALLERGIC ALVEOLITIS.

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Patients with extrinsic allergic alveolitis (EAA) have accumulations of T lymphocytes in their lungs, and CD8+ lung T cells have been implicated in the pathogenesis of EAA. In this study we analyzed the T cell receptor (TCR) Vα and Vβ gene usage of CD4+ and CD8+ lung and peripheral blood (PBL) T lymphocytes.

Materials and methods. Ten patients with clinical signs of EAA were studied (7 were exposed to mould; 3 to birds). From seven of these patients, samples were also available after clinical recovery. Lung cells, obtained by bronchoalveolar lavage (BAL), and paired PBL samples were analyzed by flow cytometry using a panel of anti-TCR V specific monoclonal antibodies.

Results. 5/7 patients had expansions, restricted to the lung, of CD8+ T cells using a particular Vα or Vβ, with a normalized V gene usage on follow-up. In four minor patients expansions using the same Vβ in both CD4+ and CD8+ subsets were identified, suggesting a possible role for superantigens in this disease.

Conclusion. In EAA we found most expansions, correlating with clinical activity, of T cells utilizing a particular TCR Vα or Vβ gene segment, to occur in the lung CD8+ T cells. This further implicates these cells in the pathogenesis of EAA. We intend to proceed studying the functional role of CD8+ T cells in EAA by stimulating T cells with the relevant antigen in vitro.

IMPAIRED INTRACELLULAR KILLING IN PHAGOCYTES OF VITAMIN A DEFICIENT RATS: one possible mechanism for the increased frequency of septic arthritis.

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Vitamin A deficiency is associated with a 3-4 times higher incidence of infectious diseases and increased mortality in children in developing countries. Accordingly we have seen that vitamin A deficient rats, inoculated i.v. with Staphylococcus aureus, develop septic arthritis with a 2 times higher frequency than the control rats.

Since phagocytes play an important role in the defence against bacterial infections, we wanted to study if the function of those cells is influenced by vitamin A deficiency.

Peripheral blood from vitamin A deficient and control rats was used to determine the percentage of phagocytic cells as well as the phagocytosis activity per cell. This was done by measuring the number of fluorocinesiothiocyanate labelled bacteria ingested by granulocytes and monocytes. To determine the capacity of intracellular killing of bacteria phagocytes from the peritoneal cavity of vitamin A deficient and control rats were recovered and incubated with staphylococcus aureus for 24 hours. Thereafter the cells were lysed and the incorporated bacteria cultured on horse blood agar plates.

The percentage of phagocytic cells in the peripheral blood of vitamin A deficient rats was about 40 % higher (p = 0.001) and the number of bacteria ingested per cell 30 % higher in vitamin A deficient than in control rats.

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The percentage of phagocytic cells in the peripheral blood of vitamin A deficient rats was about 40 % higher (p = 0.001) and the number of bacteria ingested per cell 30 % higher in vitamin A deficient than in control rats.

However, the intracellular killing capacity of the phagocytes from vitamin A deficient rats was significantly impaired (p = 0.02), since a higher number of bacteria remained alive in the phagocytes after 24 hour incubation.

The results from our study show that the relative number of blood phagocytes is increased during vitamin A deficiency. The ingestion of bacteria by these cells is not affected but the ability to kill bacteria is impaired. This might be an important mechanism explaining the increased susceptibility to infections, respectively to septic arthritis during vitamin A deficiency.
TRANSFER OF MODIFIED HUMAN IL-18 INTO TUMOR CELLS
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Two different human IL-18 cDNA constructs coding for the mature protein were prepared in order to develop a model for studying the biological effects of IL-18 release. To obtain a released form of human IL-18, a signal sequence from the related IL-1 receptor antagonist was fused to the IL-18 encoding gene construct. A panel of murine cell lines was transduced with retroviral technique and analyzed for the expression of IL-18, with (ssIL-18) or without (IL-18) a signal sequence. Cell lines transduced with IL-18 contained large amounts of the IL-18 protein whereas cells expressing ssIL-18 secreted most of the IL-18 protein. Treatment of ssIL-18 transduced cells with Brefeldin A, an inhibitor of protein transport to the endoplasmatic reticulum, induced accumulation of the protein. Release of the mature form of IL-18 from monocytes has been connected with cell death. In mouse fibroblast, melanoma, lymphoma and fibrosarcoma cell lines transduced to secrete IL-18, no accompanying cell death could be detected. Preliminary in vivo results using IL-18 and ssIL-18 transduced tumor cells demonstrated distinct regulation on tumor growth.

ANTIGEN-BINDING SITES DOMINATE THE SURFACE PROPERTIES OF IgG ANTIBODIES.
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A new technique, liquid-liquid partition chromatography in an aqueous polyethylene glycol/dextran two-phase system, was used to analyze differences in surface properties of antibodies with different antigen-binding sites. Employing well-characterized monoclonal IgG antibodies, intact as well as Fab/Fc fragments thereof, and chimeric IgG antibodies, we found a remarkable relationship between the structure of the antibody combining sites and the chromatographic behavior of the antibodies. The surface properties of IgG antibodies are dominated by those of its antigen-binding regions. In addition, the constant parts of IgG1, 2 and 4 seemed to form similar scaffolding, on to which CDRs of variable shapes and sizes are interspaced.

The surface properties of antibodies of IgG, IgM, IgA and IgE type were also compared as were the events, in terms of conformational changes, occurring upon binding of hapten by these antibodies. The importance of the observed differences in surface properties/conformational changes are discussed.

CHARACTERIZATION OF J-CHAIN EXPRESSION AND QUANTITATION OF J-CHAIN mRNA IN HUMAN B-CELL LINES.
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The J chain is a 15-kDa polypeptide present in dimeric IgA and pentameric IgM (collectively called plg). This peptide is crucial for the affinity of plg to the epithelial secretory component (SC or plg receptor) and therefore plays a key role in secretory immunity. To define at which stage of B-cell differentiation J-chain expression starts we have characterized J-chain expression in several human B-cell lines representing different stages of differentiation. Northern blot analyses were used to screen for J-chain expression and quantitative PCR was established to quantify the copies of mRNA per cell. An internal standard for the PCR reaction was constructed that utilizes the same primer pairs as the J-chain mRNA. The cell lines were phenotyped by flow cytometry for surface markers including CD38 and the integrin δ7 subunit. J chain was expressed in all cell lines except the pre-B-cell lines. This is in contrast to murine B cells where J chain is expressed only after activation. J-chain expression at the mRNA level was also detected in plasmacytoid B-cell lines that secrete IgG or IgE. The level of J-chain mRNA quantified by PCR ranged from 19000 to 350000 copies per cell. Protein and mRNA expression were well correlated.
IN VITRO MODEL OF EPITHELIAL CELL - THYMCYTO INTERACTION IN HUMAN THYMUS
A.Yarilin and N.Sharova. Laboratory of Lymphocyte cell activation and resistant to apoptosis induction. The secretion can be modulated by some hormones, cytokines and physical factors. Thymocytes adhere to HTSC cells and die by the mechanism of apoptosis during first day of co-culturing and then are eliminated. Human blood T cells and mouse thymocytes are considerably less effective in inducing HTSC cell activation and resistant to apoptosis induction.

DYNAMICS OF mRNA EXPRESSION OF IFN-γ, IL-4 AND TGF-β IN SCIATIC NERVES AND LYMPHOID ORGANS IN EXPERIMENTAL ALLERGIC NEURITIS
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Experimental allergic neuritis (EAN) is an inflammatory demyelinating disorder of the peripheral nervous system. It is a T cell mediated and cytokines including interferon-γ (IFN-γ) and transforming growth factor (TGF-β) are involved in the pathogenesis. By adopting in situ hybridization, we studied the mRNA expression of immune response up- and down-regulating cytokines in EAN. IFN-γ, interleukin-4 (IL-4) and the TGF-β were examined in the target organ, i.e. sciatic nerve, and in lymph nodes and spleen over the course of EAN. The dynamics of IFN-γ mRNA expression in the sciatic nerve followed approximately the clinical course of EAN with peak values around day 14 post immunization (p.i.), whereas IFN-γ was transcribed earlier in spleen and lymph nodes with maximum on day 7 p.i. On the contrary, transcription of IL-4 and TGF-β was only slightly enhanced in EAN, with minor fluctuations in sciatic nerves peaking on days 11 and 28 p.i. The highest numbers of TGF-β mRNA positive cells in lymph nodes were observed during clinical improvement of EAN. The data argue for a major proinflammatory role for IFN-γ and a hypothetical disease down-regulating functions for TGF-β at the target site in EAN.

MICE TRANSGENIC FOR TCR Vβ3 ARE HIGHLY SUSCEPTIBLE TO INFECTION BY ENTEROTOXIN A (SEA) PRODUCING STAPHYLOCOCCUS AUREUS
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We have previously demonstrated that staphylococcal enterotoxins contribute to the arthritis and mortality during staphylococcal infection. To further explore the relationship between staphylococcal superantigens and T cell responsiveness in the pathogenesis of hematogenic SEA producing S. aureus infection we used Tcr Vβ3 transgenic (TGVβ3) mice. TGVβ3 mice and non-TG littermates were inoculated intravenously with S. aureus AB-1 strain producing a large amount of SEA, which specifically reacts with TcR Vβ3. TGVβ3 mice were highly susceptible to the acute bacterial infection. Within 9 days after inoculation, 85% of TGVβ3 mice died compared with 31% of non-TG littermates (p<0.01). The high mortality in TGVβ3 mice was correlated with a significantly elevated bacterial burden in blood, spleen and kidney. Flow cytometry analysis displayed that inoculation with SEA-producing bacteria resulted in an initial expansion and subsequent decrease of spleen Vβ3+ CD4+ T lymphocytes in TGVβ3 mice. The In vivo kinetics of cytokine mRNA expression was revealed by an in situ hybridization technique. No significant differences of TNFα, IL-1β, IL-4 and IL-10 mRNA expression were found between the groups following inoculation with S. aureus AB-1. However, a higher expression of TNFα in TGVβ3 mice was noted throughout the course of infection compared with non-TG littermates. These findings suggest that overproduction of TNFα may play a critical role in the pathogenesis of septicemia caused by enterotoxin secreting S. aureus.

IDIOTYPE-REACTIVE T SUBSETS AND TUMOR LOAD IN MONOClonAL GAMMOPATHIES
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The presence of idiotyp-reactive T cell subsets and their relation to the tumor load were analyzed in 9 patients with monoclonal gammopathy of undetermined significance (MGUS), 12 patients with multiple myeloma (MM) clinical stage I and 9 patients with MM stage II/III. An enzyme-linked immunosorbent assay was used to identify interferon (IFN)-γ, interleukin (IL)-2- or IL-4-secreting T cells after stimulation by F(ab')2 fragments of monoclonal IgG. The response to autologous IgG was significantly higher than that induced by isotopic monoclonal IgG. Comparable results were obtained in a proliferation assay (³H-thymidine incorporation). Eight of 9 patients with MGUS, 7 of 12 patients with MM stage I and 3 of 9 with MM stage II/III had T cells secreting IFN-γ and/or IL-2 (T helper [Th] type-1 cells), whereas cells secreting both Th1 and Th2 or Th0 types of cytokines were more frequent in patients with MM, particularly in those with MM stage II/III. The number and frequency of Th1-type cells were significantly higher in MGUS patients as compared to MM stage II/III. The T cell response was inhibited by an anti-DR antibody. The results indicate that idiotype-reactive T cells of the Th1 and Th2 or Th0 subsets were present in monoclonal gammapathies, and might provide indirect evidence that idiotype-reactive Th1-type cells may have a regulatory impact on the human tumor B cells.
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