S16-19

A PUTATIVE NOVEL PROTEASE THAT IS SPECIFICALLY LOST IN TUMOR CELLS

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Eukaryotic cells that are transformed by oncogenic viruses undergo dramatic changes in their phenotype. It is likely that these changes are caused by the specific loss of proteins from the cell membrane and the cytoskeleton. To investigate these alterations at the molecular level, we prepared a subtracted cDNA library with mRNA from normal human fibroblasts and from their SV40 transformed counterparts. About one third of the clones obtained in this way showed the expected down-regulation on a Northern blot with mRNA from transformed cells. One of the clones was chosen for further investigation. It hybridized to a mRNA of 2500 nucleotides which harboured an open reading frame of more than 1000 bp. The derived amino acid sequence revealed some weak similarity to an E. coli heat-shock protease. We will study the function of this novel protein and determine its relevance in cancer.

S16-20

INDUCTION OF APOPTOSIS IN RMS CELLS THROUGH DOWN REGULATION OF PAIRED DOMAIN TRANSCRIPTION FACTORS

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The products of the developmentally regulated PAX gene family are transcription factors, which are active early in embryogenesis. Novel chimaeric genes involving either PAX3 or PAX7 have been found in the alveolar rhabdomyosarcoma (RMS), a pediatric skeletal muscle cancer. The chromosomal t(2;13) translocation product PAX3/FKHR is expressed in the alveolar RMS cell line Rh30. We also found aberrant expression of either PAX3 or PAX7 in cell lines of embryonal RMS, the second major RMS histiotype. We developed an antisense ODN strategy, designed to down regulate the PAX proteins, in order to investigate their role in this kind of tumors. We found that apoptosis was specifically induced by the down regulation of aberrantly expressed PAX3/FKHR, PAX3 or PAX7, thus suggesting that PAX proteins might play a causal role in the formation of rhabdomyosarcomas by preventing cell death.

Lymphocyte Effector Functions

S17-02

CD40-CD40 Ligand interactions are critical in T-B cooperation but not for other anti-viral CD4+ T cell functions.

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CD40-CD40L interaction is required for the generation of antibody responses to T-dependent antigens as well as for the development of germinal centers and memory B-cells. The role of the CD40-CD40L interaction in the induction of antigen-specific Th cells and in mediating Th effector function other than cognate help for B cells was studied in two infectious viral systems. Using CD40- and CD40L-deficient mice together with lymphocytic choriomeningitis virus and vesicular stomatitis virus as viral model antigens, revealed that no Ig isotype switching of virus-specific antibodies was measurable upon infection of CD40- or CD40L-deficient mice. Examination of the in vivo importance of the CD40-CD40L interaction showed that the induction of virus-specific CD4+ T cells measured by proliferation of primed virus-specific Th cells in vitro was not crucially dependent on the CD40-CD40L interaction. In addition, virus-specific Th cells primed in a CD40-deficient environment, adoptively transferred into CD40 competent recipients, were able to mediate Ig isotype switch. Th-mediated effector functions distinct from and in addition to T-B collaboration, i.e. inflammatory reactions and interleukin-dependent antiviral protection were comparable in CD40- or CD40L-deficient and normal mice.
Thus, CD40-CD40L interaction plays a crucial role in T-B interactions for Th-dependent activation of B-cells but not, or to a much lesser extent, in T-macrophage/antigen presenting cell interactions for proliferative responses of antigen-specific Th cells in vitro and for interleukin-mediated Th cell effector functions in vivo.

S17-03

CYTOTOXIC T CELL-MEDIATED APOPTOSIS

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Cytotoxic T cells (CTL) can kill their target upon TcR interaction via two independent pathways. The perforin-mediated pathway acts via the degranulation of proteases, i.e. granzymes, together with the pore-forming protein perforin to induce apoptosis. In contrast, interaction of Fas ligand present on the surface of CTLs and Fas (Apo-1) expressed on a variety of target cells mediates a still poorly defined signalling cascade in the target cell leading to death.
Using MLC-derived T lymphocytes of perforin-ko and *gld* (with non-functional FasL) mice, the molecular basis of the two killing mechanisms was compared. We show that FasL is at least in part recruited from intracellular stores. Moreover, the influence of the protooncogene *bcl-2* on the two killing pathways was examined. Examination of mice lacking both perforin and FasL reveal the existance of at least one more killing mechanism.

S17-04

Perforin- and Fas-dependent cytotoxicity in protection against cytopathic and noncytopathic viruses

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T cell dependent-cytotoxicity in vitro is mediated by two distinct mechanisms, one being dependent on perforin-expression by the effector cell and one requiring the presence of the cell death inducing molecule Fas on the surface of the target cell. It has been assumed that T cell-dependent cytotoxicity is efficient in controlling cytopathic and noncytopathic virus infections given the elimination of the infected cell is taking place at an early stage of the infectious cycle. We have tested the role of perforin- and Fas-dependent cytotoxicity in a panel of cytopathic and noncytopathic viral systems in the mouse and have found that perforin-dependent cytotoxicity is crucial for control of noncytopathic LCMV but not for control of cytopathic viruses such as vaccinia, Semliki Forest and vesicular stomatitis virus. Fas-dependent cytotoxicity, on the other hand, was not required for protection against any of the tested viruses. The implications of these findings for the understanding of the relationship between virus and the cellular immune response will be discussed.

S17-05

The DQ52 region, a complex DNA-proteins interaction locus.

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Rearrangement of the IgH gene locus always starts with a D to J joining, followed by the joining of a V gene element. Since all V(D)J rearrangement events seem to depend on the same recombinase, the ordered and allele specific targeting of this activity implies additional regulation, probably at the level of the substrate gene segments. We believe that DNA-binding factors are responsible for this fine regulation. We want to test this hypothesis using one D element (DQ52) from the IgH locus as a model. Using a gel shift mobility assay, footprinting techniques and quantitative S1 protection analysis for the transcription studies, we have identified and characterised positions within the DQ52 region that are binding sites for nuclear proteins. Interestingly, some of them are transcription factors which play a fundamental role in B cell development and more specifically in transcriptional control of the IgH locus.

S17-06

CARTILAGE OLIGOMERIC MATRIX PROTEIN (COMP): FRAGMENTS AND AUTOANTIBODIES IN SYNOVIAL FLUID OF PATIENTS WITH RHEUMATOID ARTHRITIS. Michel Neidhart[1], Nik Hauser[2], Mats Paulsson[2], Paul E. DiCesare[3], Ferenc Pataki[1], Beat A. Michel[1] and Hans-Jörg Häuselmann[1]. [1]Department of Rheumatology, Zürich; [2]Institute for Biochemistry, University of Cologne; [3]Hospital for Joint Diseases, New-York.

We evaluated whether cartilage oligomeric matrix protein (COMP) and anti-COMP autoantibodies in synovial fluids (SF) can be employed as disease markers in osteoarthritis (OA) and rheumatoid arthritis (RA). COMP was purified from human articular cartilage. Polyclonal antibodies, raised in rabbits, were used to detect the COMP levels in hyaluronidase-treated SF by competitive enzyme-linked immunosorbent assay (ELISA) and by qualitative immunoblots. IgG anti-COMP autoantibodies were detected by ELISA. In SF, compared with living controls, elevated levels of COMP were found in both patient groups. In 79% of RA patients (n = 77), the SF showed well defined low molecular weight COMP fragments (40-70 kDa). These could only be detected in 19% of OA patients (n = 47). Similarly, elevated levels of IgG anti-COMP autoantibodies were detected in RA, but much less in OA. Thus, the COMP degradation products, resulting from the action of proteases, and anti-COMP in SF seem to be promising markers in RA.

S17-07

PURIFICATION AND PHYSICO-CHEMICAL CHARACTERIZATION OF RECOMBINANT sIgA

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The hybridoma cell line ZAC3 expresses Vibrio cholerae LPS-specific mouse IgA molecules as a heterogenous population of monomeric, dimeric and multimeric forms. We describe a method combining ultrafiltration, ion-exchange chromatography, and gel permeation chromatography for the simultaneous and qualitative separation of the three molecular forms. Milligram quantities of purified IgA molecules were recovered allowing for direct comparison of the biologic properties of the three forms. In ELISA, both the dimeric and polymeric recombinant IgA antibody binds efficiently to the LPS antigen, whereas the monomer does only weakly. Secretory IgA (sIgA) can be reconstituted in vitro by combination between recombinant secretory component (SC) and purified dimeric or polymeric IgA. Binding experiments to lipid membrane-associated LPS with reconstituted sIgA and IgA indicate that both antibody species recognize LPS with identical affinity (K_{ass}: 1.2 x 10^8 M^{-1}), as determined by surface plasmon resonance. Thus, the function of SC on sIgA is not to modify the affinity for the antigen. K_{ass} falls to 6.8 x 10^5 M^{-1} when measured by calorimetry using free LPS and IgA, suggesting that the LPS environment is critical for recognition by the antibody.

S17-08

Flow cytometric and EM analysis of sub-populations of human B-lymphoblastoid cells exposed to hyperthermia.

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Hyperthermia, the exposure of patients to elevated body temperatures by microwave radiation, is a well established adjuvant to radiation therapy. To investigate the cytotoxicity of this treatment we exposed human B-lymphoblastoid (TK6) cells to various temperatures (37°C to 50°C), and for various time periods (15 min to 3 h); subsequently incubating for 23h to permit expression of apoptotic response. We observed temperature and exposure-period dependent appearance of two sub-populations of TK6 cells, which probably reflects facets of general heat-shock response. One of these sub-populations has also been observed following X-ray exposures. Because only a fraction of all cells respond in this manner, the phenomenon may be cell cycle dependent. We have isolated these sub-populations of cells using a Becton Dickinson FACS Vantage cell sorter and will present data on the flow-cytometric and electron microscopic analyses of these cells.

S17-09

Characterization of the immunological and protective properties of a long polypeptide derived from the circumsporozoite protein of *Plasmodium berghei*

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Immunization of Balb/c mice (H-2^d) with a polypeptide of 69 amino acids, which is derived from the C-terminal part of the circumsporozoite protein of *Plasmodium berghei* induces humoral as well as $CD4^+$ and $CD8^+$ cellular immune responses. Two s.c. injections of peptide PbCS 242-310 in combination with IFA or alum generate a high titer of anti-peptide antibodies which recognize also the native CS protein as detected in immunofluorescent staining of air-dried sporozoites of *P. berghei*. Furthermore, cytotoxic T lymphocytes specific for the K^d restricted peptide PbCS 252-260 as well as $CD4^+$ T cells were detected. The protective capacities of peptide PbCS 242-310 were tested by submitting immunized mice to the bites of sporozoite-bearing *A. stephensi* or *A. gambiae*; in both cases, a high level of protection was detected (70 % - 100 %). In order to determine which component(s) of the immune response induced by peptide immunization is responsible for protection, T-cell depletion were carried out *in vivo* by injection of anti-CD4 or anti-CD8 monoclonal antibodies. Protection was almost completely abrogated by anti-CD8 or by anti-CD4 treatment, indicating that both T cell subsets play an important role in the anti-parasite immunity. It remains unclear whether T cells act individually or in concerted action to eliminate parasite-infected hepatocytes.

S17-10

The functional half-life of MHC class I-restricted T cell epitopes on living cells.

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The functional $t_{1/2}$ of different complexes formed by MHC class I molecules and Ag on the surface of target cells was measured by using specific CTL clones in standard cytolysis assays. Half-life values of 5 to 10 h were obtained, that are in accordance with some previous estimations obtained from biochemical and immunochemical measurements, and that were further confirmed by using IFN-γ-production and Ca2+-mobilization assays. Moreover, these values were independent of the type of target cell, fixation of the target cells, or proteases susceptible to degrade the peptides, suggesting that the unfolding of the peptide/MHC complexes at the cell surface alone determines the $t_{1/2}$ of the CTL epitopes.

S17-11

INCREASED AGGREGATION OF HEMATOPOIETIC CELLS BY A DIVALENT METAL CATION AT CONCENTRATIONS SLIGHTLY ABOVE PHYSIOLOGICAL LEVELS

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A previous scientific collaboration using FACS analysis revealed cell aggregation of different hematopoietic cell lines by elevated concentrations of the divalent metal cation Zn2+. In a cell aggregation test performed on ice, UC cells showed aggregation within seconds at Zn2+ concentrations from 75μM to 100μM. Whereas UC cells at and below 50μM Zn2+ exhibited no aggregation. As control experiments neither 3mM of Mg2+ nor 5mM Ca2+ caused aggregation, in addition aggregation by 0.1mM Zn2+ was inhibited progressively by increasing concentrations of EDTA between 0.1 to 0.4mM. Thus aggregation of these B cells is caused by Zn2+ concentrations at and above physiological average blood concentrations of 50 μM.

Among cell adhesion receptors, eg. the integrin LFA-1 which binds ICAM-1 and -2 contains divalent cation binding repeats in its CD11 subunit. LFA-1 is expressed on most immune cells and the role of Zn2+ in comparison to Mg2+ and Ca2+ in adhesion by these receptors remains to be determined.

Garfinkel & Chaim (1980) showed correlation of decreased Zn2+ blood concentrations and many important diseases eg. cancer, arteriosclerosis and infections. This might have crucial medical importance because Zn2+ levels are frequently found below average in the Swiss population (Brenner, pers.comm).

S17-12

PHAGOCYTOSIS AND ANTIGEN-PROCESSING OF BORRELIA BURGDORFERI (Bb) BY HUMAN DENDRITIC CELLS (DC)

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Skin and blood derived DC were tested for their capacity to phagocytose and process Bb, and to activate autologous specific T cells. Electron microscopic and flow cytometric studies showed that DC engulfed Bb very efficiently. Bb were found in progressive degradation states in the lysosomal compartment, but they were also localized free and intact in the cytosol of DC. Bb antigens and MHC II molecules could be co-localized in the antigen loading compartment of DC by immuno-electron microscopy. Bb antigen loaded DC were shown to activate specific T cells by measuring cytokine gene transcription and proliferative response.

S17-13

PARALLEL TUBULAR ARRAYS (PTA) IN HUMAN NK-CELLS - A UNIQUE GRANULE FOR LYTIC PROTEINES (LP)

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Human NK-cells (CD3-,CD16+,CD56+) were enriched by negative selection. X-ray analysis and histo-chemical staining revealed elevated levels of sulfur within the PTA indicating the lysosomal character of these granules (Gr). By immuno-electron microscopy LP including perforin and granzyme B were found to be colocalized in the hexagonal tubes of the PTA. During NK-cell mediated killing and after prolonged in vitro cultivation the PTA lost the highly ordered tubes and transformed into conventional lysosomes. In parallel chondroitin sulfate (CS), a sugar known to bind LP preventing cells from self-digestion could be detected in transformed Gr but not in intact PTA.

S17-14

CD5+ AND CD5- SUBSETS OF MOUSE CD8αβ+ INTRAEPITHELIAL LYMPHOCYTES ARE DIFFERENTIALLY SELECTED IN THE INTESTINE

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We followed T cell receptor selection of the gut intraepithelial lymphocytes (IEL) of mice bearing transgenic (tg) TcRαβ specific for LCMV glycoprotein GP33, presented by H-2Db class I MHC molecules. As expected, in a non selecting genetic background (H-2Dd) no positive or negative selection of TcRαβ tg IELs expressing CD8αβ or CD8αα is observed. Positive selection of TcRαβ tg IELs expressing CD8αβ, but not of those expressing CD8αα is observed in the selecting haplotype (H-2Db). In H-2Db mice double tg for the TcRαβ and the glycoprotein GP33, tg CD8αβ are negatively selected whereas evidence for a positive selection of tg CD8αα is found. Compared to splenocytes, positive selection of TcRαβ tg CD8αβ IELs in the selecting MHC haplotype is less pronounced. To examine these differences, CD8αβ IELs were further divided into CD5+ and CD5- subsets. This fractionation revealed that the tg CD5- CD8αβ IEL are positively selected whereas the tg CD5+ CD8αβ IEL do not appear to be selected. A similar observation has been made also for the tg CD8αβ T cells in the lamina propria of the same animals. Interestingly, in mice kept under SPF conditions the ratio of CD5- : CD5+ CD8αβ+ IEL is markedly higher than in age and sex-matched mice kept under conventional conditions. Experiments are under way to determine whether most of these non-tg CD5+CD8αβ+ IEL in conventionally kept mice are derived from CD5-CD8αβ+ by expansion of cells specific for antigens the animal encounters in a non sterile environment.

S17-15

PRELIMINARY CHARACTERIZATION OF SURFACE FACTORS ON HUMAN STIMULATED T CELLS THAT ACTIVATE MONOCYTES AND FIBROBLASTS

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At the inflammatory site, infiltrating immune cells and resident tissue cells are in close proximity. We have shown direct cell-cell contact between stimulated but not resting T lymphocytes is a potent mechanism inducing pro-inflammatory factors such as matrix-destructive metalloproteinases (MMP) and cytokines in fibroblasts and monocytes. These activities are due to cell surface activating factors on stimulated T lymphocytes (SAFT). By chromatography fractionation (anion exchange and gel filtration) of solubilized membranes from stimulated HUT-78 cells, part of SAFT activity was recovered in fractions of MW≅45,000 whereas another SAFT activating fibroblasts and not monocytes was recovered at MW≅100,000. However, the latter fractions induced MMP production by fibroblasts in the absence of other product such as prostaglandin E_2. This suggests that SAFT display differential activities not only as a function of the target cell type but also as a function of the product induced. These results suggest that different types of SAFT are expressed at the surface of stimulated T lymphocytes.