Oncogenes and Tumor Suppressor Genes

S16-01

HTLV-I Tax: Role of Viral trans-activator protein in cell DNA-damage and cell cycle progression

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HTLV-I Tax is a nuclear protein that has pleiotropic effects on cellular metabolism including cellular activation and transformation. We have previously shown that expression of Tax induces the formation of Micronuclei in rapidly dividing cells[1,2]. At the molecular level, Tax function has been well-described to be mediated through CREB/ATF and NF-κB. However, it is unclear whether these are the sole cellular targets for Tax. Here we report on the identification and characterisation of three pioneer Tax-partners: TP3-1, TP18-1 and TP106-25. TP-3 is a proximal partner that binds directly to Tax. It encodes a human protein that is a suppressor for a constitutively lethal Gβ mutation in yeast . TP106-25 is a distal partner for Tax, and encodes the human analogue of the yeast MAD2 (Mitotic arrest defect) protein. TP106-25 binds TP18-1 which in turn directly contacts Tax. TP18-1 contains a promoter activating domain, and as yet we have found no relatives of this protein in existing databases. We will also present data on the role of Tax influencing cell cycle progression.

[1] Majone et al., *Virology*, 193, 456, 1993
[2] Semmes et al., *Virology*, in press, 1996

S16-02

MOLECULAR GENETICS OF ACUTE PROMYELOCYTIC LEUKEMIA

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Chromosomal tanslocations are a constant feature of leukemias and sarcomas. Cloning of the chromosome breakpoints has revealed that they frequently involve nuclear proteins, mainly transcription factors, and lead to the formation of fusion proteins. The genetics of the acute promyelocytic leukemia (APL) 15;17 translocation are of particular interest because the corresponding PML/RARα fusion protein coincides with the promyelocytic phenotype and correlates with the APL patients response to retinoic acid (RA) treatment[1-4]. Expression of the APL-specific PML/RARα fusion protein into the hematopietic U937 precursor cell line blocks differentiation by vitamin D3 and increases sensitivity to RA[5,6], suggesting that PML/RARα is involved in both the pathogenesis of APL and the response to RA. Analysis of the transactivating, dimerization and cell localization properties of the PML/RARα mutants revealed that: i) PML/RARα homodimerization correlates with RA-sensitivity; ii) PML/RARα-PML and PML/RARα-RXR heterodimers appears not to be crucial for PML/RARα activity on differentiation. We propose that the targeting of a transcription factor, RARα, with a heterologous dimerization domain, from PML, is an important mechanism underlying PML/RARα functions. Similar mechanisms might also operate in other tumor-associated fusion proteins.

Ref.: Pandolfi P.P. et al., 1992, EMBO J., 11, 1397-408. Grignani Fr. et al., 1993, Cell 74, 423-31.

S16-03

FUNCTION AND REGULATION OF RAF-1

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The serine/threonine protein kinase Raf-1 functions as a transducer in different signal pathways. Its activity is regulated by interaction with cellular proteins and lipids. 14-3-3 proteins bind to the amino- and carboxy terminus of Raf-1. In stimulated cells activated Ras can bind directly to Raf-1 and transports it to the plasma membrane for further activation. Binding of activated Ras interferes with binding of 14-3-3 zeta to the amino terminus of Raf-1. Activated Raf-1 binds to and phosphorylates MEK which in turn activates MAPK transmitting signals to transcription factors. Stimulation of the Raf-1/MAPK signal cascade can be blocked by dominant-negative mutants of Raf-1 and Raf-1 derived peptides. Raf-1 can also bind and phosphorylates the transcription factor c-Jun indicating a bypass of MAPK in certain cells.

S16-04

GENES DOWN-REGULATED IN *HRAS* ONCOGENE-TRANSFORMED CELLS: POTENTIAL SUPPRESSORS OF MALIGNANT TRANSFORMATION

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We have identified a number of genes (*H-rev*) that are physiologically expressed, but down-regulated in the presence of mutant *RAS* oncogenes in preneoplastic rat 208F and mouse NIH/3T3 fibroblasts and in transformed cell lines derived from them. In phenotypic revertants, *H-rev* mRNA levels are partially restored to pretransformation levels. The genes encode extracellular matrix components (e.g. type I collagen), the matrix-modifying enzyme lysyl oxidase (Lox), the angiogenesis inihibitor thrombospondin, and two novel proteins (Ril, Hrev107). The Ril protein appears to bind to α-actinin and other yet unknown proteins. In density-arrested fibroblasts, most of the Hrev107protein, otherwise cytosolic, is associated with the membrane fraction. We and others have demonstrated transformation-suppressing activity of *lox*, α-actinin and *H-rev107* genes in different recipient cell lines. These results suggest that oncogenic Ras signalling results in the coordinated down-regulation of multiple transcriptional targets. Conversely, suppression of *RAS* transformation is probably mediated by simultaneous up-regulation of critical target genes.

S16-05

PROBING P53 STRUCTURE IN YEAST: BASIC SCIENCE AND CLINICAL APPLICATIONS.

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We have developed a simple functional assay for p53 mutation in which human p53 expressed in *S.cerevisiae* activates transcription of the *ADE2* gene: yeast colonies containing wild type p53 are white and colonies containing mutant p53 are red. Since this assay tests the critical biological function of p53 it can distinguish inactivating mutations from functionally silent mutations. We show that this assay can be used to detect mutations in blood samples, tumours and cell lines

We have identified residues in p53 important in contacting DNA by selecting mutants with an increased affinity for DNA. Mutations at amino acids 120, 121, 277 and 279 selected in this way affect not only affinity but also sequence specificity. This supports the crystallographic identification of amino acids 120, 277 and 280 as major groove DNA contact residues. The unusual properties of our mutants may allow us to dissect downstream pathways activated by p53, and data on the biological activity of the mutants will be presented.

S16-06

EXPRESSION OF V-SRC IN THE MOUSE BRAIN LEADS TO A NEOPLASTIC PHENOTYPE.
ANIMAL MODEL FOR ASTROCYTOMAS.

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Src family kinases activate a signal transduction pathway that culminates in the transcription of c-myc and finally in DNA synthesis and mitosis. To direct the expression of the v-src kinase in the mouse brain, the v-src gene was set under the regulatory control of a modified murine glial fibrillary acidic protein (GFAP) gene. GFAP is a specific marker for astroglial cells in the mammalian CNS. For the generation of transgenic mice, a GFAP construct was used that harbors the entire exon/intron structure of the mouse GFAP gene and the 5' regulatory sequences with the basal promotor plus an additionally introduced SV40 (simian virus 40) late gene splice and polyadenylation signal in exon one. Three lines of transgenic mice were derived. Transgene expression was demonstrated by RT-PCR. GFAP staining of brain sections from one 25 week old transgenic animal showed strong reactivity mainly in the thalami and midbrain. Multiple preneoplastic lesions and multifocal astrocytomas were found. We hope that these animals will be suitable for the study of multi-step tumorigenesis in gliomas.

S16-07

IDENTIFICATION AND CLONING OF A NOVEL PROTEIN IMPLICATED IN CANCER

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Extracellular matrix forms the glue which keeps cells together to form tissues. It interacts through specialized membrane structures also with cytoskeletal elements of individual cells. Cancer cells, compared to normal cells, often show altered behavior in tissues due to modified compositions of these linking membrane structures. We tried to identify such structures by raising Mabs against human placental membrane proteins. We selected a Mab recognizing an antigen possibly involved in metastasis in the B16 F1/ F10 mouse melanoma system. Incubation of the melanoma cells with the specific Mab prior to tail vein injection reduced the lung colonization about 85%. The antigenic molecule in B16F1 cells could be identified as a 150 kDa (p150) membrane associated protein. New Mabs raised against isolated mouse p150 reconfirmed the results obtained with the original Mab. P150 expression studies on various cell lines showed a positive correlation with the transformed phenotype as well as a negative correlation with the differentiation status. Furthermore 14 investigated human breast carcinoma pairs (carcinoma versus normal tissue) showed in more than 80% a strong positive correlation as well. P150 peptide sequences enabled us to clone an ORF of 1343 aa. Data bank searches revealed in 5 cases molecules with significant degrees of homologies. 4 of these proteins show a common overall organization. However p150 is the largest and only member yet containing a hydrophobic stretch of 70aa at the corboxy terminus. Currently we are trying to evaluate the diagnostic or prognostic use of p150 expression in human cancer.

S16-08

DEREGULATED EXPRESSION OF PAX-5 IN HUMAN MEDULLOBLASTOMAS AND ECTOPIC EXPRESSION IN MOUSE BRAIN

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Genes involved in developmental control, such as the *homeobox* and *paired box* genes, can act as oncogenes. We have shown by RNase protection analysis that the expression of PAX-5 is deregulated in a large series of medulloblastomas. In situ hybridization analyses show a tight link between proliferation and PAX-5 expression, and an inverse correlation with differentiation.To further investigate the oncogenic potential of PAX genes in medulloblastoma, we have generated high titer retroviral vectors for the murine PAX-5 and PAX-6 genes under control of the PGK-1 promoter. Target cells infected *in vitro* with infectious supernatant produced high levels of PAX-5 and PAX-6 protein. The presence of functional protein was confirmed by gel retardation assays. Primary mouse embryonic neuroectodermal cultures were infected with these vectors and transplanted into adult hosts in a brain reconstitution model. In addition, packaging cells were injected into the proliferating murine neonatal cerebellum. Continuing expression of PAX-5 *in vivo* was demonstrated in transplanted packaging cells by *in situ* hybridization. The impact of expression of PAX-5 and PAX-6 on differentiation and neoplastic transformation in these models will be discussed.

S16-09

ELEVATED FREQUENCY OF P53 INDEPENDENT APOPTOSIS IN ATAXIA TELANGIECTASIA HOMOZYGOUS AND HETEROZYGOUS CELL LINES

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The response of ataxia telangiectasia homozygous and heterozygous cell lines to ionising radiation was compared to normal lymphoblastoid cells using the comet assay. AT cells exhibited a significantly increased proportion of DNA breaks than could be expected after radiation alone. Fragmentation was active and could be inhibited by caffeine, cycloheximide and EDTA. Cytospin preparations revealed the presence of apoptotic bodies especially in the AT cell line. Thus fragmentation appeared to be induced by the activation of an apoptotic pathway. AT homozygous cells exhibited the highest apoptotic frequency compared to control and AT heterozygous cells. Since the radiation induced p53 response is markedly delayed in AT cells compared to controls (the protein being undetectable until about 4 hours post irradiation), lymphoblastoid cells of AT patients appear to be defective in the appropriate induction not only of a p53 dependent, but also a p53 independent apoptotic pathway.

S16-10

IDENTIFICATION OF BRCA1 MUTATIONS IN A SELECTED SERIES OF BREAST CANCER PATIENTS

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Mutations in the BRCA1 gene have been associated with the development of early onset breast and/or ovarian cancer. In an effort to determine how many women with early onset disease could be accounted for by mutations in the BRCA1 gene we investigated the BRCA1 gene using the newly described protein truncation test (PTT).

From a series of 70 women who fulfilled our selection criteria we identified four separate mutations, all of which resulted in a premature stop codon as detected by the PTT assay. Expanding the pedigrees of the affected patients revealed that all of them had a family history of disease consistent with the notion that mutations in the BRCA1 gene appear not to be associated with sporadic disease. All mutations were found towards the 3 ' end of the gene adding support to their being a phenotype/genotype gradient within the BRCA1 gene as only breast cancer was observed in the respective families.

S16-11

CORRELATION BETWEEN THE DEVELOPMENT OF EXTRACOLONIC MANIFESTATIONS IN FAP AND MUTATIONS IN THE APC GENE.

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Familial adenomatous polyposis is an autosomal dominantly inherited disorder with a high predisposition to colorectal cancer. Germ-line mutations in the adenomatous polyposis coli (APC) gene are responsible for the development of disease.

The APC gene was investigated in 31 unrelated polyposis coli families by Single Strand Conformation Polymorphism (SSCP) and the Protein Truncation Test. Twenty three germ-line mutations were identified which gave rise to a variety of different phenotypes. Typical disease symptoms were observed in families who harboured mutations between exon 4 (codon 169) and codon 1393 of exon 15. Mutations beyond codon 1403 were associated with more varied phenotype with respect to the development of extracolonic manifestations.

S16-12

The Receptor for Advanced Glycosylated Endproducts of proteins (RAGE) and the human homologue to the Rat Matrix Glycoprotein SC1, (MAST9): Two Tumor Suppressor Candidates?

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The gene that encodes the Receptor for Advanced Glycosylated Endproducts of proteins (RAGE) is expressed in normal lung tissue but down-regulated in Non-Small Cell Lung Carcinoma (NSCLC) at both the transcriptional and translational levels. RAGE a new member of the immunoglobulin superfamily of cell surface molecules shares homologies to DCC (Deleted in Colon Carcinoma). Antisense studies using the PC12 rat phaeochromocytoma cell line indicate RAGE's involvement in cell adhesion. Immunostaining showed RAGE expression in normal epithelial cells of pulmonary mucosal glands. The second gene MAST9 is also down-regulated in NSCLC. Sequence analysis of the cDNA reading frame revealed significant homology to the rat matrix glycoprotein SC1. The C-terminus is similar to the Secreted Protein Acidic and Rich in Cysteins (SPARC) an extracellular matrix molecule that is involved in cell growth regulation. We started with transfection assays with in order to evaluate MAST9's regulatory effects on cellular growth.

S16-13

ANALYSIS OF THE TUMOR SUPPRESSOR PROTEIN P53 IN RAT CARDIOMYOCYTES

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A successful reactivation of cell division in the case of permanently postmitotic heart cells would allow subsequent application in organ repair e.g. after myocardial infarction. Unlike in skeletal muscle, division and proliferation of differentiated ventricular heart muscle cells in the adult mammals are not activated after injury. Knowledge of the mechanisms that control the cardiac cell cycle are almost non-existent but it is possible that so called tumor suppressor proteins play an important role, prominent ones being pRb and p53.
We found the tumor suppressor protein p53, which frequently shows point mutations causing loss of cell cycle control, in a remarkable amount attached to the actin stress fibres in adult rat cardiomyocytes in culture. In freshly isolated and still dividing neonatal cardiomyocytes, however, no or only little p53 protein could be demonstrated. 2-D gel electrophoresis in addition suggests a hypophosphorylated state of p53 in the adult cardiomyocytes, which would reflect a possible mechanism for preventing adult heart cells from proliferation.

S16-14

MOLECULAR AND FUNCTIONAL ANALYSIS OF THE EPSTEIN-BARR LMP1 ONCOGENE PROMOTER IN LYMPHOPROLIFERATIVE DISEASES.

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The expression of Epstein-Barr virus LMP1 oncogene is tightly regulated by viral and cellular factors in a tissue dependent manner. In human B-cells its expression is induced by the EBV encoded transcription factor EBNA2. LMP1 is also present in the majority of nasopharyngeal carcinoma tumour cells and in Reed-Sternberg cells from Hodgkin's lymphoma, in which the only EBV nuclear antigen detected is EBNA1. Our aim was to test if mutations affecting the expression of the LMP1 gene were present in lymphoproliferative disorders. For this purpose we have characterised the LMP1 promoter region from seven cases including two aggressive Hodgkin's disease and two atypical lymphoproliferative syndromes. Our results show that the sequences of LMP1 promoter region (-298 to + 29 relative to the transcription startsite) diverged up to 9.3 % when compared to the prototype EBV strain B95-8. The cAMP responsive-like element (CREB) located at position -37 to -44 that was shown to be critical for the control of LMP1 expression is found to be mutated in 3/7 cases. Functional analysis in the human epithelial cell line 293 using the firefly luciferase reporter gene demonstrate that the mutations within the CREB site leads to a 75% diminished reporter activity . Our analysis suggest that, in lymphoproliferative disorders, variants with a weaker promoter activity exhibit low but persistent LMP1 oncogene expression.

S16-15

TIME COURSE OF AXOTOMY-INDUCED APOPTOTIC CELL DEATH IN FACIAL MOTONEURONS OF NEONATAL WILD TYPE AND BCL-2 TRANSGENIC MICE.

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In neonatal animals, axotomy of facial motoneurons induces cell death. The time course of axotomy-induced motoneuron death was studied using the TUNEL technique. Following a facial nerve lesion in two days old pups, motoneurons died within 5 days. Labelled motoneurons (with DNA fragmentation) are detected as early as 16 hours after the lesion and a peak of dying motoneurons is observed 28 hours after the lesion. The kinetic of appearance of DNA breaks was correlated with the loss of cresyl violet stained motoneurons. Moreover, labelled facial motoneurons were also observed in the contralateral side of the lesion suggesting that a spontaneous apoptotic cell death occurs during the post-natal period. In transgenic mice, whose motoneurons overexpress Bcl-2, no TUNEL-labelled cells were detected in the lesioned and unlesioned side. These results suggest that 1) the timing of appearance of dying motoneurons is relatively fast since 80 % of the apoptotic cells were detected 28 hours after the lesion and 2) the overexpression of Bcl-2 can block the DNA fragmentation generated by axotomy or during a naturally occuring cell death process.

S16-16

POLYOMAVIRUS MIDDLE-T ANTIGEN ASSOCIATES WITH THE KINASE DOMAIN OF SRC-RELATED TYROSINE KINASES

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Middle-T antigen, the oncogene product of mouse polyomavirus, associates with and activates the cellular tyrosine kinases c-Src, c-Yes, and Fyn. Mutational analysis showed that the kinase domain of c-Src, including the carboxy-terminal regulatory 'tail', is sufficient for association with middle-T. Moreover, we found that Hck, another member of the Src kinase family, does not bind middle-T, while chimeric kinases consisting of the amino-terminal domains of c-Src fused to the kinase domain of Hck or the amino-terminal domains of Hck fused to the kinase domain of c-Src, as well as Hck mutated at its carboxy-terminal regulatory residue, tyrosine 501, were found to associate with middle-T. These results suggest that in Hck the postulated intramolecular interaction between the carboxy-terminal regulatory tyrosine and the SH2 domain prevents association with middle-T. This intramolecular interaction apparently also limits the ability of c-Src to associate with middle-T, since removal of the SH2 or SH3 domain increases the efficiency with which middle-T binds c-Src.

S16-17

CHARACTERIZATION OF MAMMARY GLAND DEVELOPMENT IN FOS-DEFICIENT MICE

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Fos is a major component of transcription factor AP-1. AP-1 is active during normal proliferation of mammary epithelial cells at puberty and in preneoplastic and neoplastic lesions suggesting a role for AP-1 during proliferative phases in the gland (1). Interestingly, a requirement for elevated Fos (AP-1) was also found in the early period of post-lactational involution when a majority of secretory epithelial cells die by programmed cell death (2, 3). We studied the develoment of the mammary gland in Fos-deficient mice. The epithelial compartment of these animals is characterized by a severe lack of terminal end bud formation during puberty resulting in a very limited number of epithelial structures in the glands of adults. The studies are extended by determining steroid hormone levels in the blood, and by measuring the expression of different AP-1 components (mainly JunD which is the predominant partner of Fos in the mammary gland) and potential target genes of *fos* (AP-1) during postnatal development by in situ hybridization and immunohistochemstry.

(1) Marti, A. *et al.* (1995) Cell Death Diff. *2*: 277-283.
(2) Jaggi, R. *et al.* (1996) J. Dairy Sci. *(in press).*
(3) Feng, Z. *et al.* (1995) J. Cell Biol. *131*: 1095-1103.

S16-18

CHARACTERIZATION OF GENES DIFFERENTIALLY EXPRESSED IN HUMAN PRIMARY MYOBLASTS AND EMBRYONAL RHABDOMYOSARCOMA (eRMS)

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Using a substractive hybridization method, 48 cDNAs have been cloned. These are expressed in human primary myoblasts but downregulated in the eRMS cell line RD. Twenty nine sequences were identified as coding for previously known gene products, while 19 code for unknown proteins. Unknown clones, downregulated in the RD cell line were chosen for extensive Northern blot analysis. The obtained results suggest that several isolated clones may have an important role in the determination or maintenance of the normal phenotype and additionally considered as candidate tumor suppressor genes. To gain further insight to the function of some clones they will be overexpressed in RD cells using a viral based delivery system. A modified form of the retroviral vector has been constructed to achieve higher expression of the desired genes.

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.