INVITED SPEAKER ABSTRACTS

SA1 REGULATION OF TRANSCRIPTION DURING T CELL DIFFERENTIATION

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CD4 T lymphocytes mediate their biologic effects through the synthesis and secretion of cytokines. The nature of the cytokine, which is produced, determines the function of a given CD4 T cell and the outcome of the immune response. Naive CD4 T cells produce predominantly interleukin 2 and utilize it to facilitate clonal expansion and hence the increase in the magnitude of the specific immune response to a given pathogen. CD4 T cells differentiate into effector cells which secrete either pro-inflammatory cytokines such as IFNγ and TNF or anti-inflammatory cytokines which can also potentiate the humoral immune response such as IL4. The molecular basis for the differential expression of cytokines during T cell activation and development is unknown. At least part of the regulation is mediated at the level of transcription. To study these processes we have generated reporter transgenic mice in which a given transcription factor drives a reporter gene, in our case the luciferase. Transgenic mice in which this gene is driven by the inducible factors AP-1, NFAT and NFκB have been generated as well as two analogous reporters for elements carrying CREB elements which are part of the IFNγ gene promoter. These reporters appear to express specifically and provide a useful readout not only for the transcription factors themselves, but for the signaling machinery that leads to their activation. In addition, transgenes encoding dominant negative and constitutively active signaling molecules have been used to study the small G proteins and kinases which mediate the activity of these transcription factors. Finally, gene targeting has been used to eliminate a number of the kinases on these signaling pathways. The role of these components in the activation, differentiation and apoptosis of lymphocytes will be discussed.

SA2 THE FUNCTIONAL SPECIALIZATION OF CHROMATIN: ARCHITECTURAL AND REGULATORY ROLES FOR HISTONES

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Current models for nucleosome structure (1) allow the accommodation of regulatory proteins into comparable architectures (2). Other regulatory proteins direct the assembly of canonical nucleosomes at a particular sites that can activate or repress transcription (3, 4). Transcriptional co-activators and co-rerepressors can also function through the directed modification of nucleosomal structure (5, 6). These experimental observations lead to a view of chromatin structure in which architectural specificity is an integral component of transcriptional regulation.

1. Pruss, D., Hayes, J.J. and Wolffe, A.P. (1995) Nucleosomal anatomy. BioEssays 17 161-170
2. Wolffe, A.P. and Pruss, D. (1996) Deviant nucleosomes. Trends in Genetics 12 58-62
3. Wolffe, A.P. (1994) Transcription: in tune with the histones. Cell 77 13-16
4. Wolffe, A.P. (1994) Architectural transcription factors. Science 264 1100-1101
5. Wolffe, A.P. and Pruss, D. (1996) Targeting chromatin disruption: transcription regulators that acetylate histones. Cell 84 817-819
6. Wolffe, A.P. (1996) Histone deacetylase: a regulator of transcription. Science 272 371-372

SA3 CHROMATIN CONFIGURATION AND LINEAGE COMMITMENT

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T cell differentiation is the result of sequential expression of genes which is concomitant with lineage commitment. Gene expression is linked with conformational changes of chromatin. Thus, expressed genes have a DNaseI hypersensitive configuration whereas non expressed genes are insensitive to the action of this nuclease.

Decision not to express a gene sometimes is taken at the chromatin packaging level and this event can have a stochastic nature. Thus, some cells can silence a gene whereas others within the same lineage can express this particular gene. "Closed" or "Open" chromatin configuration seems to be associated with these decisions respectively. The repercussions of such decisions on lineage commitment on the developing T cell and the DNA sequences involved will be discussed.

SB4 RETROVIRUSES FOR GENE THERAPY

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We have demonstrated that the sensitivity of retroviruses to human serum is controlled by the expression of alpha1-3 galactosyl sugar epitopes. We have now made high titre packaging cells producing viruses resistant to human serum; their use in in vivo gene therapy will be discussed.

We are also attempting to retarget retroviruses to novel surface receptors using an insertion point in the MLV envelope which allows incorporation of an additional receptor binding domain. Our results with chimeric envelopes expressing single chain antibodies will be discussed in the context of other published work in this field.

Finally, our strategies using retroviruses for tumour gene therapy will be presented. These include ex vivo modification of tumour cells which are being used in a melanoma vaccine clinical trial, direct gene delivery to peritoneal tumours and targetting retroviral delivery to tumour vasculature cells.

SB5 ARTHRITIS GENE THERAPY

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Gene therapy holds much promise as a novel treatment for arthritis and related conditions (reviewed in Curr. Opin. Rheumatol. 8:230, 1996). Although this can be approached in a number of different ways, we have concentrated on delivering to the synovial lining of joints genes which encode secreted, anti-arthritic proteins. This can be achieved both by direct *in vivo*, and indirect *ex vivo*, strategies. Most progress has been made using a retrovirus (MFG-IRAP) in the *ex vivo* transfer of a cDNA encoding the human interleukin-1 receptor antagonist (IL-1Ra). Transfer of this gene has a beneficial effect in animal models of arthritis and no adverse side effects of the procedure have been noted. Based on extensive pre-clinical testing, a human protocol trial was proposed and approved by RAC, FDA and an independent monitoring board that we established to oversee this trial. *Ex vivo* transfer of the IL-1Ra gene to human knuckle joints was accomplished on 17/7/96, thus initiating the first human trial of gene therapy for arthritis or any other chronic, non-lethal disease.

SB6 Self proteins as targets for tumour-reactive cytotoxic T lymphocytes (CTL).

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CTL can efficiently control the malignant growth of tumour cells in experimental mice and probably also in humans. The antigens recognised by tumour-reactive CTL have remained elusive for a long time. However, in the past few years the specificity of human and murine tumour-reactive CTL has been determined in many laboratories. It has become clear that CTL not only recognise strictly tumour-specific antigens such as the products of mutated oncogens or proteins encoded by transforming viruses, but also unaltered self proteins expressed at elevated levels in tumour cells.

Mdm-2 is a zinc finger protein that can bind to p53 and neutralise its transcriptional activity. Mdm-2 and p53 are both frequently expressed at elevated levels in transformed cells. We have investigated in a mouse model whether it is possible to raise CTL against mdm-2. Since T cell tolerance is self MHC-restricted, we exploited the allo-restricted CTL repertoire to raise CTL against the murine mdm-2 protein. Thus, BALB/c mice ($H\text{-}2^d$) were used as donors to generate CTL specific for an mdm-2 derived peptide presented in the context of $H\text{-}2K^k$ class I molecules. These CTL killed K^k-positive tumour cells but not K^k positive dendritic cells. When BALB/c ($H\text{-}2^d$) derived CTL clones were injected into C57BL/6 ($H\text{-}2^b$) recipient mice, they delayed but did not prevent the growth of K^k positive tumour cells. In contrast, the CTL clones completely prevented tumour growth in BALB/c x C57BL/6 recipients. These data indicate that allo-restricted CTL clones can display potent anti-tumour activity, provided that host immune responses against these CTL are suppressed. Therefore, allo-restricted CTL clones may efficiently control tumour growth in immunosuppressed cancer patients.

SEe109 T CELL SURVEILLANCE AND IMMUNE ESCAPE IN HTLV-I INFECTION/

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Infection with the human T cell leukaemia virus HTLV-I causes no serious disease in the great majority (95%) of infected people. In a minority of cases, infection results in either a T cell malignancy, or a chronic inflammatory disease of one or more organs. We are interested in the part played by the immune response to HTLV-I in determining the different outcomes of infection. There is a powerful, chronically activated cytotoxic T lymphocyte (CTL) response to the Tax protein of HTLV-I in most people infected with the virus. The CTL select variant sequences of Tax which escape immune recognition, and interfere with the recognition of the wild-type protein. This positive selection process is more efficient in healthy HTLV-I carriers than in patients with HTLV-I-associated myelopathy (tropical spastic paraparesis, HAM/TSP), the most common chronic inflammatory disease associated with HTLV-I. The mean provirus load is more than ten times greater in patients with HAM/TSP than in healthy carriers of HTLV-I. We conclude that anti-Tax CTL play an important part in limiting the rate of replication of HTLV-I. We suggest that the outcome of infection with HTLV-I is primarily determined by the CTL responsiveness of the individual: low immune responders control virus replication inefficiently, and are more likely to develop chronic inflammatory disease. We propose to test this hypothesis in a population genetic study in an area of high HTLV-I seroprevalence in southern Japan.

SEe110 EVASION OF CD8+ T LYMPHOCYTES BY HERPES SIMPLEX VIRUS.

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Previously, we demonstrated that the HSV ICP47 protein blocks the Transporter associated with Antigen Presentation (TAP) which translocates antigenic peptides across the ER membrane. Analyses of ICP47/TAP interactions have indicated that ICP47 binds to TAP with high affinity, blocking peptide binding but not ATP binding and remains stably associated with the cytosolic domain of TAP. Since ICP47 is one of the first proteins produced in infected cells, it can inhibit the association of antigenic peptides with MHC class I proteins, so that peptides are not presented to anti-HSV CD8+ T cells. ICP47 does not block the mouse TAP, complicating in vivo experiments. However, a mutant HSV, R3631, which does not express ICP47 as well as the neighbouring US11 protein, was defective in its ability to replicate in the nervous system of mice. This was related to CD8+ T cells because R3631 killed mice that were depleted of CD8+ T cells. CD8+ T cells are known to control HSV in sensory ganglia and the CNS. Other viruses with deletions in the ICP47 gene (not affecting US11) or the US11 gene also displayed reduced pathogenesis and this was increased in mice lacking CD8+ T cells. Therefore, we suspect that both ICP47 and US11 collaborate to produce resistance to CD8+ T cells in mice, although ICP47 does not block the mouse TAP.

SEe111 HIV ESCAPES FROM CYTOTOXIC LYMPHOCYTES

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HIV infection is initially controlled by a strong cytotoxic T lymphocyte response. Infecting virus is brought down to low levels and the CTL response persists until very late stages of infection. We have shown that antigen specific CTL are present at up to 1% of peripheral blood lymphocytes during this phase. Despite this strong T cell response, the virus infection is poorly controlled in many patients and ultimately escapes in virtually all. One means by which virus can escape from T cell control is by mutation. Escape mutant viruses have been demonstrated in acute primary HIV infection, during middle phase of asymptomatic infection and associated with the onset of AIDS. Complete escape to fixation has been observed in several patients followed over months or years. In other examples at single time points, prevalent virus mutants that cannot be recognised by circulating CTL have been seen. Taken together, these provide strong evidence that HIV can escape from CTL control by mutation. It is often observed that there are CTL present directed at several epitopes. This may arise from a complex pattern of mutation and escape with shifting immunodominant CTL responses. Some HLA types, by chance, select parts of the virus that can vary more readily than others. This may account for some of the HLA associations with slow or rapid progression.

SIi112 TARGETED ANTI-CANCER THERAPY USING A CHIMERIC ANTI-CD20 ANTIBODY IN THE TREATMENT OF NON-HODGKIN'S B CELL LYMPHOMA

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The CD20 antigen expressed on B cells provides a promising target for therapy of non-Hodgkin's B cell lymphoma. The CD20 antigen expressed at high density does not shed from the cell surface and does not internalize after binding to antibody. The accessibility of the lymphatic compartment to antibodies and the sensitivity of lymphoma cells to lysis by host effector mechanisms render targeting with antibodies a promising approach. We constructed a high affinity mouse/human chimeric antibody (IDEC-C2B8) that is expressed at high levels in CHO cells. IDEC-C2B8 binds human C1q and mediates cell killing through complement (CDC) and human effector cell (ADCC) mechanisms. When administered to cynomolgus monkeys, IDEC-C2B8 caused a marked B cell depletion in peripheral blood, bone marrow and lymphatic tissue. In a Phase I trial of IDEC-C2B8, 15 patients receiving intravenous infusions of 10 to 500 mg/m^2 were rapidly and specifically depleted of CD20 positive cells in peripheral blood within 72 hours. Tumor responses occurred in 6 of 9 patients who received >100 mg/m^2. In a subsequent Phase II trial, 37 patients with advanced-stage low-grade, or follicular lymphoma received four weekly infusions of 375 mg/m^2. An objective response rate of 50% was observed in 34 evaluable patients. Toxicities were generally mild and were limited to rare cases of Grade IV thrombocytopenia in some patients receiving their first infusion. These encouraging results have led to the initiation of a pivotal Phase III trial, which has recently completed patient enrollment. The results of the pivotal trial will be presented.

SIi113 SINGLE CHAIN Fv ANTIBODIES FOR TARGETING CANCER THERAPY.

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The use of antibody libraries expressed in filamentous bacteriophage (phage) makes it possible to examine examine great diversity in the antibody repertoire. Single chain Fv (scFv) antibodies selected from such libraries and produced in prokaryotic systems overcome several problems of specificity, affinity, safety, stability and yield. A clinical imaging study of a phage-derived scFv directed against carcinoembryonic antigen in patients with colorectal carcinoma has shown favourable imaging and safety characteristics. This forms a basis for using the scFvs as the targeting component of fusion proteins of antibody and effector molecule. As an example we have constructed an anti-CEA-carboxypeptidase G2 (CPG2) fusion protein for use in antibody-directed enzyme prodrug therapy. This has been expressed in *E.coli* and shown to localise functioning CPG2 selectively to colon carcinoma xenografts in nude mice at concentrations necessary for prodrug activation selectively in the tumour. This technology can produce antibody-based pharmaceuticals with many favourable characteristics and a wide range of applications.

SIi114 TARGETING T-CELLS TO CANCER USING CHIMERIC RECEPTORS

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In order to expand the spectrum of recognition of effector lymphocytes and redirect them to predefined targets, we have endowed T cells with antibody specificity using chimeric receptor genes consisting of antibody variable regions combined with cell activating domains. Several basic designs have been constructed. The first was composed of the two chimeric T cell receptor chains (cTCR) in which the constant regions of the T cell receptor chains were combined with the VH and VL of specific monoclonal antibody. The second design employed a single-chain Fv of antibody as the recognition unit, linked to the signal transducing γ or ζ subunits of the FcR or CD3 (scFvR). The use of the scFvR design enables the use of more efficient gene delivery systems such as retrovectors. Furthermore, it allows the use of several lymphocyte triggering receptor subunits, thus extending the potential of the chimeric receptor (T-Body) approach to different cell types. In a third design, we bypassed the TCR complex and connected the extracellular antibody recognition unit through CD4 or CD8 transmembranal stretch directly to intracellular protein-tyrosine kinases (PTK). Following transfection or transduction into T cells, the chimeric genes were expressed as functional surface receptors and conferred non-MHC restricted, antibody-type specificity on the recipient cells. Upon encountering antigen (either immobilized or displayed on target cells), the chimeric receptors triggered T cell activation. Syk was found to be the preferential intracellular kinase in the scFv-PTK chimeric receptor design and could transmit signals leading to phosphorylation, IL-2 release and specific target cell cytolysis. When Fv of anti-human carcinoma antibodies such as the anti-HER2 or anti-folate binding protein antibodies were employed, cytotoxic lymphocytes (either CTL or NK) harboring the chimeric genes could specifically lyse the corresponding human tumor target cells.