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 reporter 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-repressors 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.
BioEssays 17 161-170
Genetics 12 58-62
13-16
1100-1101
transcriptional regulation that acylate histones. Cell 84 817-819
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 DNaesI 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 packing 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
sera is controlled by the expression of alpha1-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
targeting 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-arthritis proteins. This can be achieved both by direct
in vivo, and indirect ex vivo, strategies. Most progress has been made using a
retrovirus (MFG-IKAP) in the ex vivo transfer of a CDA encoding the human
interleukin-1 receptor antagonist (IL-1ra). Transfer of this gene may
have 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 recognized by tumour-reactive CTL have
remained elusive for a long time. However, in the past few years the specificity of
human and mouse tumour-reactive CTL has been determined in many laboratories. It has
become clear that CTL not only recognize strictly tumour-specific antigens such as
the products of mutated oncogenes or proteins encoded by transforming viruses, but also
self-encoded proteins expressed at elevated levels in tumour cells.
Many-2 is a zinc finger protein that can bind to p53 and neutralise its transcriptional
activity. Many-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 many-2. Since T cell tolerance is self MHC-restricted, we exploited the allo-
restricted CTL repertoire to raise CTL against the murine many-2 protein. Thus, BALB/c
mice (H-2d) were used as donors to generate CTL specific for an many-2 derived peptide
presented in the context of H-2Kc class I molecules. These CTL killed K-positive
tumour cells but not K+ positive adenocarcinoma cells. When BALB/c (H-2d) derived CTL
cloned against many-2 were injected into C57BL/12 (H-2d) recipient mice, they deleted but did not
prevent the growth of K+ positive tumour cells. In contrast, the CTL clones completely
prevented tumour growth in BALB/c x C57BL/10 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. These allo-restricted CTL
clones may efficiently control tumour growth in immunosuppressed cancer patients.
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 role 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 primary HTLV-I infection, despite the virus. The CTL target variant sequences of Tax which escape immune recognition, and interfere with the recognition of the wild-type protein. The positive selection pressure 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 CTLs play an important role 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.**


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, R363, which does not express ICp47 as well as the neighbouring US1 protein, was defective in its ability to replicate in the nervous system of mice. This was related to CD8+ T cells because R363 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 US1) or the US1 gene also displayed reduced pathogenesis and this was increased in mice lacking CD8+ T cells. Therefore, we suspect that both ICp47 and US111 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 viral escape can occur 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.

**SEe112**

**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 lymphoma cell to antibodies and the sensitivity of lymphomas to the antibody are believed to be mediated by cell host effector mechanisms. Therefore targeting with antibodies a promising approach. We constructed a high affinity mouse/human chimeric antibody (IDEC-2B8) that is expressed at high levels in CHO cells. IDEC-2B8 binds human IgC1q and mediates cell killing through complement (CDC) and human effector cell (ADCC) mechanisms. When administered to cyclophosphamide treated mice, IDEC-2B8 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/m² 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/m². In a subsequent Phase II trial, 37 patients with advanced-stage low-grade, or follicular lymphoma received four weekly infusions of 375 mg/m². 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.

**Sli112**

**TARGETING T-CELLS TO CANCER USING CHIMERIC RECEPTORS.**


In order to expand the spectrum of recognition of effector lymphocytes and redirect them to predefined targets, we have developed T cell receptors specific for tumours. The first was a chimeric T cell receptor (CTC) in which the constant regions of the T cell receptor chains were combined with the VI and VH of specific monoclonal antibodies. The second design employed a single-chain Fv unit linked to the signal transducing y or C gamma subunits of the FeR or CD3 (scFv). The use of the scFv design enables the use of the high avidity antibody gene delivery systems such as retargeting. Furthermore, it allows the use of several lymphocytes triggering signals in addition to that of the chimeric receptor (TCR) approach to different cell types. In a third design, we bypassed the TCR complex and connected the extracellular antibody recognition unit with CD4 or CD8 transmembranal stretch directly to immunoreceptor tyrosine-based protein tyrosine kinases (PTK). Following transfection or transduction into T cells, the chimeric genes were expressed as functional receptor engendering monofunctional, antibody-specificity on the recipient cells. Upon encountering antigen (either immunostimulated or displayed on target cells), the chimeric receptor is preferentially activated, depending on the nature of antigen, and the targeted triggering 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 cytotoxicity. When Fv of anti-human carcinoma antibodies such as the anti-HER2 or anti-folate-binding protein antibodies were employed, cytototoxic lymphocytes (cytotoxic CD8 and NK cells) harboring the chimeric genes could specifically lyse the corresponding hormone target cells.

**Sli113**

**TARGETING T-CELLS TO CANCER USING CHIMERIC RECEPTORS**


In order to extend the spectrum of recognition of effector lymphocytes and redirect them to predefined targets, we have developed 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 two chimeric T cell receptor chains (CTC) in which the constant regions of the T cell receptor chains were combined with the VI and VH of specific monoclonal antibodies. The second design employed a single-chain Fv unit linked to the signal transducing y or C gamma subunits of the FC receptor or CD3 (scFv). The use of the scFv design enables the use of the high avidity antibody gene delivery systems such as retargeting. Furthermore, it allows the use of several lymphocytes triggering signals in addition to that of the chimeric receptor (TCR) approach to different cell types. In a third design, we bypassed the TCR complex and connected the extracellular antibody recognition unit with CD4 or CD8 transmembranal stretch directly to immunoreceptor tyrosine-based protein tyrosine kinases (PTK). Following transfection or transduction into T cells, the chimeric genes were expressed as functional receptor engendering monofunctional, antibody-specificity on the recipient cells. Upon encountering antigen (either immunostimulated or displayed on target cells), the chimeric receptor is preferentially activated, depending on the nature of antigen, and the targeted triggering 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 cytotoxicity. When Fv of anti-human carcinoma antibodies such as the anti-HER2 or anti-folate-binding protein antibodies were employed, cytotoxic lymphocytes (cytotoxic CD8 and NK cells) harboring the chimeric genes could specifically lyse the corresponding hormone target cells.

**Harrogate, 10-13 December 1996**

**Strategies used by viruses to evade their hosts' immune system**

**Targeting cytotoxic immunotherapy**