HLA transgenic mice – a tool for dissecting predisposition to autoimmunity

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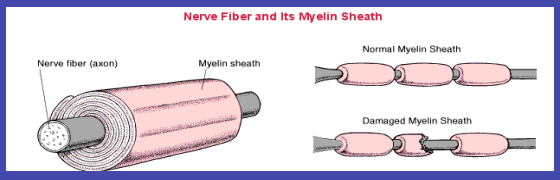
Some examples

- Classic, textbook, autoimmune diseases defined by clinical tests of autoreactivity:
  - systemic lupus erythamatosis (SLE)
  - thyroiditis (Grave's Disease, Hashimoto's)
  - myasthenia gravis
  - Goodpasture's disease
- Diseases of presumed autoimmune aetiology
  - multiple sclerosis
  - type I diabetes
  - Crohn's disease

Multiple sclerosis as an autoimmune disease



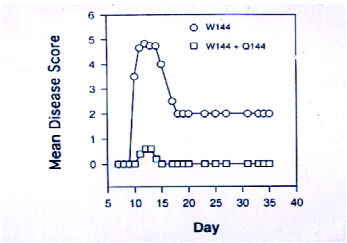
An immunologist's view of MS:

- CNS axons are myelinated by glial cells, optimising nerve conduction
- T lymphocytes specific for self myelin proteins cross the blood brain barrier and destroy the myelin sheath, causing loss of conductivity and leading to axonal loss.

Evidence for immune system involvement (MS)

- Oligoclonal immunoglobulin bands seen in cerebrospinal fluid
- Therapeutic immunosuppression (e.g. cyclosporin)
- Genome scans indicate multiple genes, strongest contribution coming from the HLA immune response genes, particularly HLA-DR15, DQ6
- Presence of T cell infiltrates at the site of plaques
- Disease is mimicked by experimental allergic encephalomyelitis, in which T cells are necessary and sufficient to induce disease
- During the course of MS and EAE autoreactive T cell responses are observed against myelin peptides

A detailed understanding of TCR/peptide/MHC interactions in autoimmunity should facilitate the design of effective immunotherapies



Genetics AND environment have a role in susceptibility - any experimental model must take this into account

- Autoimmune diseases tend to show low concordance between monozygotic twins e.g. around 30% in MS
- Much epidemiological evidence for change in risk with human migration e.g. relatively high incidence of MS in second generation Asians in UK
- Autoimmune events can quite specifically follow infection e.g. reactive arthritis

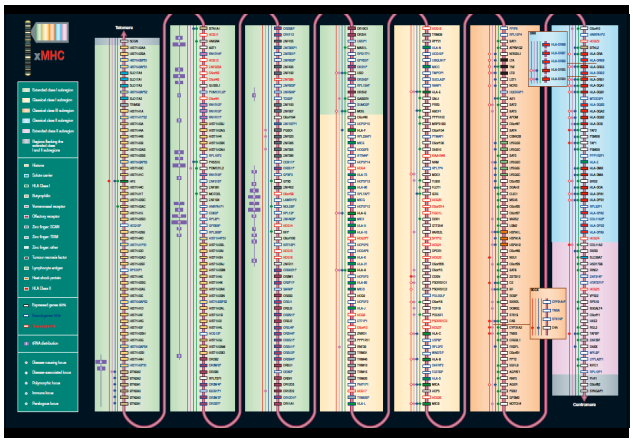
HLA disease associations

Disease	Allele	Relative risk
Ankylosing spondylitis	B1*2701	69
Coeliac disease	DQB1*0201	52
Goodpastures disease	DRB1*1501	9
	DRB1*0701	0.3
Graves disease	DRB1*0301	4
IDDM, juvenile onset	DRB1*0301/0401	23
	DQB1*0302	11
	DQB1*0602	0.1
IgA nephropathy	DRB1*0301	7
Multiple sclerosis	DRB1*1501/DQB1*0602	6
Narcolepsy	DQB1*0602	56
Pemphigus vulgaris	DQA1*0201/DQB1*0503	100
Rheumatoid arthritis	DRB1*0401	3
SLE	DRB1*0301	6

For many autoimmune diseases, the strongest genetic association is with HLA genes

These are the human immune response genes, regulating presentation of antigen to T cells

So, by Occam's razor, maybe the association reflects how potently a self antigen(s) is presented to T cells

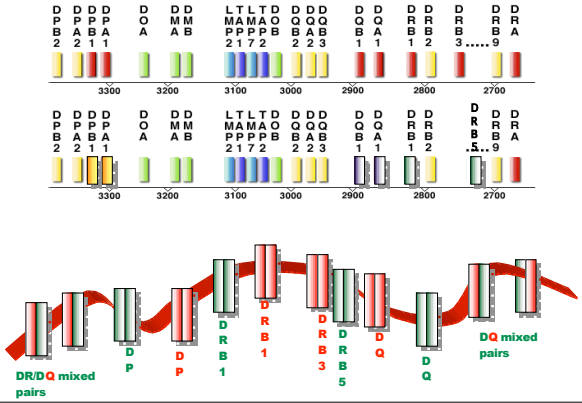


The MHC:
comprises 421 loci,
of which 252 are classified as expressed genes,
139 as pseudogenes

Table 3 | **A minimal set of immune-system genes in the human xMHC**

Category	Genes
Antigen processing/ presentation	*HLA-A, -B, -C, -DMA, -DMB, -DOA, -DOB, -DPA1, -DPB1, -DQA1, -DQA2, -DQB1, -DQB2, -DRA, -DRB1, -DRB3, -DRB4, -DRB5; PRSS16; PSMB8, PSMB9; TAP1, TAP2, TAPBP; UBD*
Immunoglobulin superfamily	*AGER; BTN1A1, BTN2A1, BTN2A2, BTN2A3, BTN3A1, BTN3A2, BTN3A3, BTNL2; C6orf25; MOG*
Inflammation	*ABCF1; AIF1; DAXX; IER3; LST1; LTA, LTB; NCR3; TNF*
Leukocyte maturation	*DDAH2; LY6G5B, LY6G5C, LY6G6D, LY6G6E, LY6G6C*
Complement cascade	*BF; C2, C4A, C4B*
Non-classical MHC class I	*HLA-E, HLA-F, HLA-G; HFE*
Immune regulation	*NFKBIL1, RXRB, FKBPL*
Stress response	*HSPA1A, HSPA1B, HSPA1L; MICA, MICB*

Most of these genes have established functions for innate or adaptive immunity; genes with remote links have been excluded. Some genes have been included because they are related by sequence to a known immune gene family but the precise function of these is still to be determined. The largest class of immune system genes is involved with antigen processing and presentation, and includes classical class I and II molecules, as well as some of the antigen processing machinery for loading peptides onto class I molecules. xMHC, extended major histocompatibility complex.



Analysis of human immune responses is complex and therapeutic intervention in these responses can be high-risk. 'Humanized (HLA, TCR) transgenics offer a reductionist bridge

The rough guide to making a transgenic

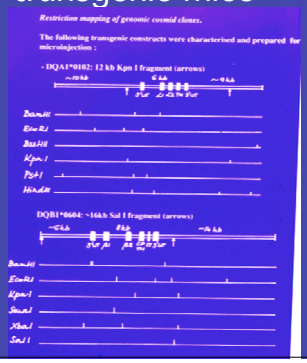
- Clone the gene of interest, being careful to characterise and encompass any upstream and downstream regulatory DNA sequences necessary for correct tissue expression
- Prepare a high quality prep of this DNA
- Meanwhile, conduct timed matings of some mouse breeding pairs, allowing the harvesting of single-cell, fertilised oocytes
- Using a glass microinjection needle, inject some DNA into the pronucleus of the oocyte
- Surgically re-implant the injected oocytes into the oviduct of a pseudopregant foster mother (prepared by mating with a vasectomised male)
- Wait for litters and genotype by PCR for gene of interest

What are the uses of HLA transgenic disease models?

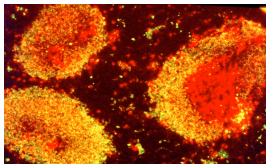
- Predict epitopes relevant to human disease
- Work out mechanisms in disease pathogenesis
- Create a more relevant, 'humanised' test-bed for therapeutics

Generation of relevant HLA class II transgenic mice

- Need to clone and characterize large, full-length genomic clones encompassing upstream and downstream regulatory sequences

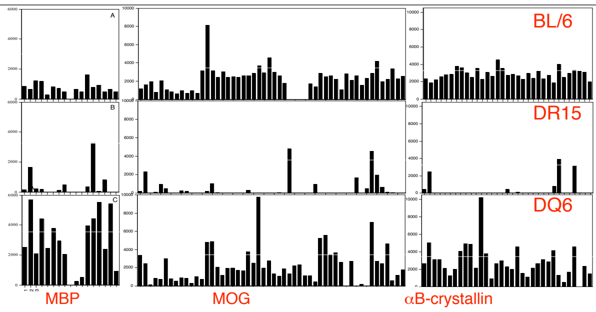


Genomic clones give faithful tissue distribution in HLA-DR transgenic mice



- HLA-DR1 cosmid clone gives characteristic splenic pattern of colocalisation with IgM around follicles and staining of DCs in T cell area

Predicting HLA-DR and HLA-DQ restricted T cell epitopes from human myelin that should be relevant to immune responses of MS patients:



HSCH immunized HLA-DQ6 transgenics (lower panel), HLA-DR15 transgenics (middle panel) or controls (upper panel) to identify HLA-restricted T cell epitopes in overlapping peptide panels of MBP (left), MOG (centre) and αB-crystallin (right)

Model HLA-restricted human responses in HLA transgenic mice

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Volume 181 March 1995 867–875

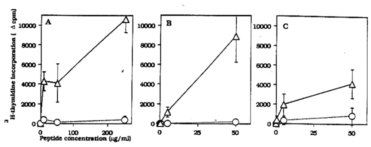


Figure 4. T cell proliferation to antigenic peptides in HLA-DR transgenic mice. Responses of popliteal lymph node cells were analysed in mice which had been immunized with (A) HA 307-319, (B) MBP 139-154, or (C) tetanus toxoid 830-843. Responses in HLA-DR transgenic mice are shown as open triangles and in non-transgenic littermates as open circles. Results are shown as the mean Δcpm ± SE from three mice per group assayed individually. Background counts of T cells and medium were <1,000 cpm.

HLA transgene dependent disease induction

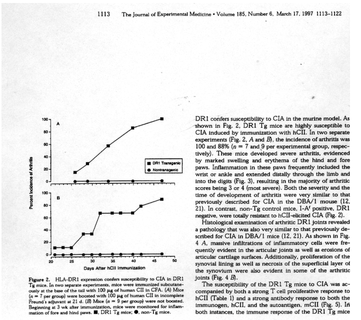


Table 1. HLA class II transgenic models of autoimmune responses.

HLA allele	Disease modelled	Antigen studied	Findings	Additional comments	R
HLA-DRB1*0401	Rheumatoid arthritis	Type II collagen	261-273 immunodominant epitope	Also found in RA patients' responses	[4
HLA-DRB1*0401	Rheumatoid arthritis	Type II collagen	Immunisation can cause HLA dependent polyarthritis	Induce with whole CII or 261-273. Similar findings in HLA-DR1 and HLA-DR4. Epitope overlaps mouse H2-A^q epitope.	[5
HLA-DRB1*0401	Rheumatoid arthritis	HCgp39	3 immunodominant epitopes identified. Differential epitopes between susceptibility (0401) and control (0402) alleles	Epitopes confirmed in studies of human patient T cell responses	[4
HLA-DRB1*0401	Type I diabetes	GAD65	Identification of GAD65 274-286, and 115-127 as DR4 restricted epitopes		[4
HLA-DRB1*0401	Type I diabetes	GAD65	6 immunodominant epitopes identified	Epitopes confirmed in DR4 patients	[4
HLA-DQB1*0302	Type I diabetes	GAD65	GAD521-535 implicated as epitope associated with susceptibility	Comparisons on susceptible and non-susceptible mice and affected and unaffected twins.	[4
HLA-DQB1*0302	Type I diabetes	GAD65	Adoptive transfer of disease with GAD 247-266 and 509-528 specific lines	Recipients needed pre-treatment with streptozotocin to generate islet insult	[5
HLA-DQB1*0302	Type I diabetes	GAD65	GAD65 121-140, 201-220, 231-250, and 471-490 identified as DQ8 epitopes.		[4
HLA-DQB1*0302	Type I diabetes	GAD65	Three immunodominant regions identified, 51-120, 111-180, 521-585, as well as definition of common core motif	Epitopes confirmed in DQ8 patients	[4
HLA-DQB1*0302	Type I diabetes	Proinsulin	Differences in proinsulin epitope recognition between DQ8 and DQ6 mice		[5
HLA-DRB1*0401	Type I diabetes	Preproinsulin and proinsulin	Immunodominance of 73-90 peptide	Sequence spans region that is proteolytically destroyed during maturation of the insulin molecule	[4

HLA-DQB1*0302	Type I diabetes	endogenous	Spontaneous diabetes if mice are crossed with RIP-B7-1 line	Study showed HLA specificity since mice expressing the diabetes protective DQB1*0601 allele did not develop disease	[50]
HLA-DRB1*0101	Multiple sclerosis	MBP	Responses to MBP 139-154 reiterate those of DR1 patients	Responses were unaffected by the presence or absence of a human CD4 transgene	[38]
HLA-DRB1*0401	Multiple sclerosis	MBP	EAE like symptoms resulting from immunisation with PLP 175-192	MBP 87-106 induced little response or disease. Evidence for impact of HLA expression in general rather than a disease associated allele.	[60]
HLA-DRB1*1501	Multiple sclerosis	MBP	Used transgenics as a tool for generation of a 1501/MBP85-99 specific monoclonal		[61]
HLA-DRB1*1502	Multiple sclerosis	PLP	DR15-restricted PLP 95-116 specific mouse cells transfer CNS disease	Mimics 95-116 response of patients. Model utilises the 1502 allele showing a possible association in Japanese patients rather than the 1501 allele most commonly associated with disease.	[49]

Identification of Novel Human Aggrecan T Cell Epitopes in HLA-B27 Transgenic Mice Associated with Spondyloarthropathy[1]

Wolfgang Kuon,[2]* Maren Kuhne,* Dirk H. Busch,‡ Pamir Atagunduz,* Martina Seipel,* Peihua Wu,* Lars Morawietz,† Gabriele Fernahl,† Heiner Appel,* Elisabeth H. Weiss,§ Veit Krenn,† and Joachim Sieper*

The pathology of ankylosing spondylitis, reactive arthritis, and other spondyloarthropathies (SpA) is closely associated with the human leukocyte class I Ag HLA-B27. A characteristic finding in SpA is inflammation of cartilage structures of the joint, in particular at the site of ligament/tendon and bone junction (enthesitis). In this study, we investigated the role of $CD8^+$ T cells in response to the cartilage proteoglycan aggrecan as a potential candidate autoantigen in BALB/c-B27 transgenic mice. We identified four new HLA-B27-restricted nonamer peptides, one of them (no. 67) with a particularly strong T cell immunogenicity. Peptide no. 67 immunization was capable of stimulating HLA-B27-restricted, $CD8^+$ T cells in BALB/c-B27 transgenic animals, but not in wild-type BALB/c mice. The peptide was specifically recognized on P815-B27 transfectants by HLA-B27-restricted CTLs, which were also detectable by HLA tetramer staining ex vivo as well as in situ. Most importantly, analysis of the joints from peptide no. 67-immunized mice induced typical histological signs of SpA. Our data indicate that HLA-B27-restricted epitopes derived from human aggrecan are involved in the induction of inflammation (tenosynovitis), underlining the importance of HLA-B27 in the pathogenesis of SpA. *The Journal of Immunology,* 2004, 173: 4859–4866.

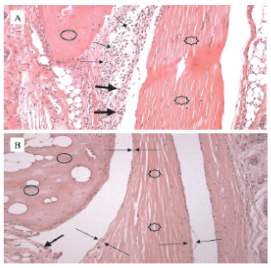


FIGURE 10. Example of a tenosynovitis in BALB/c-B27 transgenic mice upon immunization with peptide no. 67. *A*, Sagittal cut through the knee joint of BALB/c-B27 transgenic mouse immunized with peptide no. 67. The tendon sheath (thin arrows) is enlarged edematose and infiltrated by lymphocytes. The stroma cells (fibroblasts) are activated (broad arrows). Stars, Tendon; circle, bone. *B*, Equivalent cut of the knee joint of a control BALB/c-B27 mouse, injected only with CFA/IFA, showing neither signs of teanzynovitis nor other inflammation. Thin arrows, Tendon sheath; broad arrows, synovialis; stars, tendon; circle, bone. H&E staining; original magnification, ×200.

Because these are polygenic diseases of complex aetiology involving both genetic and environmental factors, there are (almost) no examples where simply expressing the predisposing HLA molecule causes spontaneous disease.

Some further nudge is required, such as:

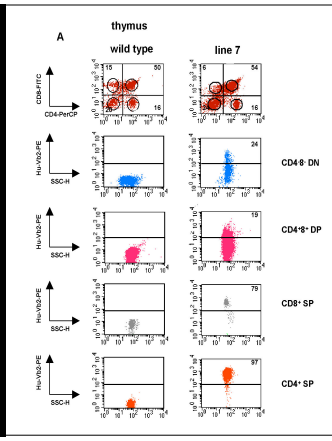
a local inflammatory insult from expression of a cytokine or co-stimulatory molecule

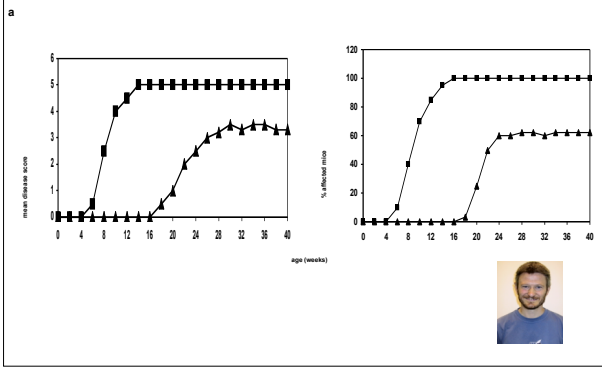
injection of self-peptide

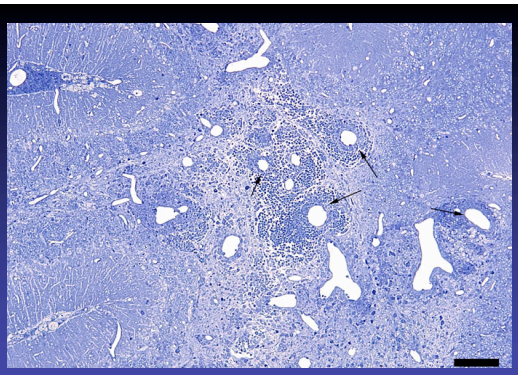
expression of a cognate, pathogenic, autoimmune T cell receptor.

We generated a spontaneous, humanised transgenic model of MS by co-expression of HLA-DR15 and a human T cell receptor specific for HLA-DR15/MBP 85-99

Transgenic expression of human Vβ2 TCR against HLA-DR15/MBP 85-99







Extensive perivascular cuffing in medullary centre of the cerebellum

In Vivo Evidence for the Contribution of Human Histocompatibility Leukocyte Antigen (HLA)-DQ Molecules to the Development of Diabetes

By Li Wen,* F. Susan Wong,‡ Jie Tang,* Ning-Yuan Chen,* Martha Altieri,* Chella David,‖ Richard Flavell,‡§ and Robert Sherwin*

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http://www.jem.org

-A really significant paper on HLA transgenics for analysing autoimmunity

In vivo evidence for the contribution of human histocompatibility leukocyte antigen (HLA)-DQ molecules to the development of diabetes.

Wen L, **Wong FS**, **Tang J**, **Chen NY**, **Altieri M**, **David C**, **Flavell R**, **Sherwin R**.
Section of Endocrinology, Department of Internal Medicine, the. Yale University Shool of Medicine, New Haven, CT

Although DQA1*0301/DQB1*0302 is the human histocompatibility leukocyte antigen (HLA) class II gene most commonly associated with human type 1 diabetes, direct in vivo experimental evidence for its diabetogenic role is lacking. Therefore, we generated C57BL/6 transgenic mice that bear this molecule and do not express mouse major histocompatibility complex (MHC) class II molecules (DQ8(+)/mII(-)). They did not develop insulitis or spontaneous diabetes. However, when DQ8(+)/mII(-) mice were bred with C57BL/6 mice expressing costimulatory molecule B7-1 on beta cells (which normally do not develop diabetes), 81% of the DQ8(+)/mII(-)/B7-1(+) mice developed spontaneous diabetes.

Spontaneous diabetes in HLA-DQ8/RIP.B7 transgenics

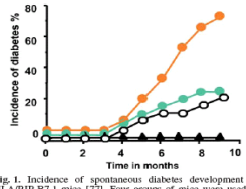

Fig. 1. Incidence of spontaneous diabetes development in HLA/RIP-B7.1 mice [77]. Four groups of mice were used in the study. Orange circles: DQ8/RIP.B7; turquoise circles: DR4/RIP.B7; white circles: DQ8DR4/RIP.B7; black triangles: DQ6/RIP.B7. Number of mice per group: 12 to 27. The mice were housed in specific pathogen-free conditions and spontaneous diabetes development was monitored weekly by urine glucose and confirmed by blood glucose (>13.9 mmol/l). The experiments ended when the mice were over 9 months old

Diabetologia (2004) 47:1476–1487
DOI 10.1007/s00125-004-1505-5

Diabetologia

Review

What can the HLA transgenic mouse tell us about autoimmune diabetes?

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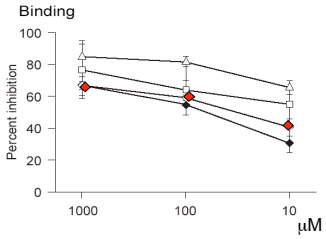
Abstract

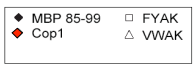
Type 1 diabetes mellitus is a polygenic disease strongly associated with the class II molecules DR3, 4 and the linked DQ2, 8 alleles. These molecules play an important role in presentation of peptide antigens after intracellular processing to CD4 T lymphocytes. A number of in vitro approaches have been used to elucidate the molecular basis for the association of particular HLA alleles with susceptibility to or protection from Type 1 diabetes mellitus. These have focused on the structure of the antigen-presenting molecules, together with their peptides. Binding studies, peptide elution, molecular modelling and crystallisation of the peptide MHC complex have between them made it possible to define the peptide-binding regions and to examine the stability of binding of peptides from putative autoantigens. It is difficult to study the role of these molecules in vivo in humans, and HLA transgenic mice have been generated to overcome this problem. Studies of mice expressing the HLA class II alleles associated with diabetes have shown that the presence of HLA molecules alone does not cause disease except in the presence of an islet "insult", even when this "insult" would in itself be insufficient to precipitate disease in the absence of the HLA class II transgene. HLA transgenic mice offer a way to elucidate the in vivo role of these molecules, and could help the development of targeted immunotherapy.

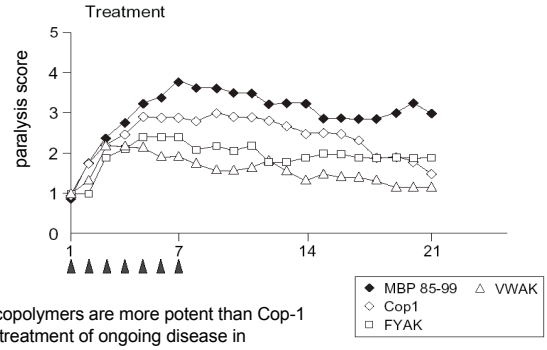
Keywords Autoimmunity · HLA · Transgenic mouse · Type 1 diabetes mellitus

Developing new, more relevant therapeutics to treat disease:



Novel coplymers show better inhibition of MBP 85-99 binding to DR15 than Cop1





Novel copolymers are more potent than Cop-1 for the treatment of ongoing disease in line 8 mice

Lecture summary

- In many (or most) autoimmune diseases, the strongest genetic association is with the genes in the HLA region
- Several other genes will also be involved, also environmental factors (infection)
- The HLA associations are generally assumed to relate the classical function of these gene products in antigen presentation to T cells
- So called 'humanised' HLA transgenic mice thus offer the chance of mimicking this event in an in vivo model
- This is important for epitope prediction, analysis of pathogenic mechanisms and design of therapeutics
- In (almost) no case is simple expression of the predisposing HLA transgene sufficient to give disease - some other trigger is needed. This may involve immunising with self antigen, expression of the cognate TCR, or expression of a cytokine or co-stimulatory molecule.