Activation of autoreactive T cells by peptides from human pathogens

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Activation of autoreactive T cells is a necessary – but not sufficient – step in the development of T cell mediated autoimmunity. Autoreactive T cells can be activated by viral and bacterial peptides that meet the structural requirements for MHC molecule binding and T cell receptor recognition. Due to the degenerate nature of MHC class II molecule binding motifs and a certain degree of flexibility in T cell receptor recognition, such microbial peptides have been found to be quite distinct in their primary sequence from the self-peptide they mimic.

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Abbreviations

CNS	central nervous system
EAE	experimental autoimmune encephalomyelitis
EBV	Epstein–Barr virus
hsp	heat shock protein
MBP	myelin basic protein
MS	multiple sclerosis
NOD	nonobese diabetic
TCR	T cell receptor
v	variable region

Introduction

Over the past few years, a new view of T cell recognition has emerged. TCRs were previously thought of as simple 'on/off' switches that were highly specific for a single 'foreign' peptide. Numerous studies have now demonstrated different functional outcomes resulting from the engagement of the same TCR by different peptide ligands depending on whether a peptide acts as an agonist, partial agonist or antagonist of a TCR [1]. At the same time, it has been shown that several peptides that have relatively little primary sequence homology to each other can all act as agonists of the same TCR [2,3]. The activation of autoreactive T cells by such pathogen-derived peptides that mimic self-peptides has been termed 'molecular mimicry' [4,5]. The emerging complexity of TCR recognition has important implications for understanding both self-tolerance and the pathogenesis of human autoimmune diseases. This review will discuss the structural requirements for the activation of autoreactive T cells by microbial peptides in the development of human autoimmune diseases.

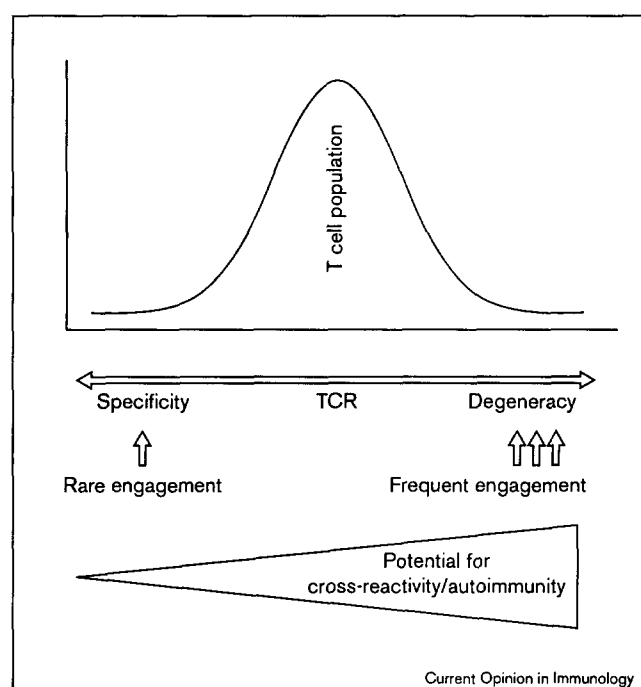## Specificity versus the degeneracy of T cell receptor recognition

MHC molecule binding of peptides is highly degenerate; a given MHC molecule will bind peptides from the majority of antigens when a set of overlapping antigen-derived peptides is tested. Peptide elution studies have demonstrated that hundreds of different peptides are bound by a single MHC class II molecule [6,7]. For the majority of MHC class II molecules, every position in the peptide can be substituted by one of a few or many different amino acids. T cell recognition, however, is much more specific as a T cell clone will not cross-react with the majority of 'control' antigens tested. Addition or removal of a single methyl or hydroxyl group at a primary TCR contact residue can result in the complete loss of T-cell recognition for some T cell clones. Changes at other positions are tolerated to a certain extent, however [8].

TCRs are generated by random rearrangements of germline encoded segments during fetal development. In contrast to antibodies, there is no affinity maturation process that improves the fit of a TCR for a given MHC molecule–peptide complex during an ongoing immune response. The specificity of the adult TCR repertoire is a balance between two possible extremes: highly specific TCRs that require particular amino acids at many positions of the MHC molecule-bound peptide and highly degenerate TCRs that only require certain sequence features in the MHC molecule-bound peptide and therefore cross-react with many different microbial peptides. The obvious disadvantages of these extremes are that the highly specific TCRs may never be engaged in an immune response (i.e. the respective microbial peptide sequence may not exist) and that the highly degenerate TCRs may cross-react with many self-peptides and cause autoimmunity. The balance between these two extremes is likely to be the result of the thymic selection process: firstly, negative selection will delete the T cells that have a high affinity for the MHC molecule (regardless of its peptide content) and that are likely to be highly cross-reactive, and secondly, a failure to undergo positive selection will eliminate the T cells that have a too low an affinity for MHC molecules.

Experimental data indicate that T cell clones differ in the degree of specificity versus degeneracy of peptide recognition. Human myelin basic protein (MBP)-specific T cell clones with degenerate peptide recognition were found to cross-react with a number of microbial peptides whereas T cell clones that were more specific cross-reacted only with one or two microbial peptides (M Martin and K Wucherpfennig, unpublished data). Each TCR

peptide-contact residue could be substituted by at least one structurally related amino acid for T cell clones with degenerate peptide recognition; clones that were more specific required amino acid identity at the primary TCR peptide-contact residues (M Martin and K Wucherpfennig, unpublished data). These data suggest that the mature T-cell repertoire represents a spectrum of T cell clones in terms of the degree of specificity/degeneracy of peptide recognition (Figure 1). According to this model, T cell clones with degenerate peptide recognition are more frequently engaged in an immune response and are more likely to induce an autoimmune response due to cross-reactivity between a microbial and a self-peptide.

Figure 1

Schematic depicting the distribution of peripheral T cells regarding the degree of specificity/degeneracy of their TCR. Degenerate T cells will be engaged by peptide–MHC molecule complexes more frequently and have a higher potential for causing autoimmunity than more specific TCRs.

Methods used to isolate T cell clones may determine from which part of the spectrum T cell clones are derived. Early cloning of T cell lines or the generation of T cell lines under limiting dilution conditions may favor sampling from different parts of the spectrum. Long-term selection of polyclonal populations under limiting concentrations of antigen is likely to favor the outgrowth of T cells that have the highest degree of specificity for a given MHC molecule–peptide complex.

Structural basis of specificity and degeneracy in T cell receptor recognition

The crystal structure of the complex of a human TCR, an HTLV-I tax peptide and HLA-A2 has been solved

[9**]; this structure provides important insights into the structural basis of TCR specificity and degeneracy. The HTLV-I tax specific TCR was oriented diagonally over the MHC molecule–peptide complex, with the CDR1 and CDR3 loops from both the α and β TCR chain variable regions contacting the peptide. The same orientation of the TCR on the MHC molecule–peptide complex was observed for a murine MHC class I restricted TCR [10**], suggesting that this binding mode is general. The peptide binding surface of the TCR was found to be remarkably flat, with the exception of a central pocket that occupied the central residue (Y5: in single-letter amino acid code) of the tax peptide. This pocket was created by the CDR3 loops of both $V\alpha$ and $V\beta$. The small size of the contact surface between the TCR and the tax peptide ($326\,\text{Å}^2$) and the fact that substantial contacts were made only to the peptide residues Y5 and Y8 indicates that there are limits placed on the specificity of a TCR. When Y5 was substituted by alanine (Y5A) the peptide still formed TCR–peptide–HLA-A2 complexes that crystallized and acted as a partial agonist [9**].

Recognition of peptide sequences that are strikingly different by the same TCR has been observed for several alloreactive T cell clones [11,12*,13]. For example, the well characterized murine cytotoxic T cell clone 2C, that reacts against H-2L^d in complex with the endogenous p2Ca peptide LSPFPFDL [14], also recognizes another endogenous peptide dEV8 EQYKFYSV presented either by self H-2K^b or another allo-MHC molecule H-2K^{bm3} [12*], and the synthetic peptide SIYRYYGL bound by the self-MHC H-2K^b [13,15]. All peptides, although distinct in their sequence, are recognized by the same TCR with high specificity, although with a different affinity [12**].

There are two structural explanations for this phenomenon. First, two peptide–MHC molecule complexes in which the peptides differ in their primary sequence may form similar TCR-accessible surfaces in terms of volume, charge, and hydrophobicity, allowing the TCR to engage those complexes in a similar way. Second, there may be a certain degree of flexibility in the conformation of the TCR CDR loops, allowing a TCR to specifically bind to different MHC molecule–peptide surfaces [16*]. The crystal structures of such different TCR–peptide–MHC molecule complexes are required to answer this question.

Activation of human autoreactive T cell clones by viral and bacterial peptides

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS) myelin. Genome wide analyses have demonstrated that multiple loci confer susceptibility to MS, with the MHC locus on chromosome 6 being a primary susceptibility locus [17–19]. Among MHC class II haplotypes, the HLA-DR2 haplotype (DRB1*1501) confers the highest risk for the development of MS. Migration studies have also implicated environmental exposure as a risk factor [20]. Despite the

evidence that environmental exposure is important, no single pathogen has been shown to be responsible for the development of the disease.

The T cell response to myelin antigens, in particular MBP and proteolipid protein (PLP), has been studied by a number of groups. The immunodominant epitopes of these antigens have been defined and the MBP region amino acids 85–99 (MBP[85–99]) was found to be immunodominant in patients carrying the HLA-DR2 (DRB1*1501) haplotype [21–23]. MBP(85–99)-specific T cells were found to be clonally expanded in MS patients [24], supporting their role in the pathogenesis of the disease.

The recognition of HLA-DR2-presented MBP(85–99) by autoreactive T cell clones from MS patients has been studied extensively and illustrates several aspects of specificity/degeneracy in T cell recognition. Analysis of the fine specificity of MBP(85–99)-specific T cell clones, by using single amino acid analog peptides, revealed a high degree of specificity toward some ('major') TCR contact residues combined with a certain degree of degeneracy toward other ('minor') TCR contacts. For example, the addition of a single hydroxyl group (F91Y) completely abrogated T cell activation by some clones, whereas changes at other positions (e.g. I95A) had little effect [25,26]. Certain substitutions even enhance HLA-DR2 binding (T97A) or TCR recognition (these mutations differ between T cell clones), representing so called 'superagonists'. Such substitutions can compensate for other substitutions that are suboptimal, resulting in multisubstituted peptides that have a similar efficacy as the native antigen [25,27]. In addition to such 'additive' effects, 'complementary' substitutions have been described in which a T cell clone does not respond to a single amino acid substituted peptide (F91Y or K93R) but only to the double-substituted peptide (F91Y K93R) [28•].

The HLA-DR2 binding/TCR recognition motif of MBP(85–99) was used to identify cross-reactive peptides from human pathogens via a protein data base search. Seven viral and a single bacterial peptide were found that stimulated at least one out of the seven MBP-specific T cells clones studied ([3]; see Table 1). The microbial peptides were quite distinct in their sequence from the MBP peptide and each other as only the primary TCR contact residues (either F91 or K93) were conserved. Presentation of microbial peptides by infected cells was examined for the Epstein–Barr virus (EBV) DNA polymerase peptide. Reactivation of the Epstein–Barr virus (EBV) lytic cycle by phorbol esters resulted in the activation of an MBP-specific T cell clone that cross-reacted with the EBV peptide, but not in the activation of a control clone that only recognized the MBP peptide. T cell activation was not observed with an MHC-mismatched B cell line and was blocked by an anti-DR antibody [3].

Comparison of the fine specificity of the MBP-specific autoreactive T cell clones also demonstrated obvious differences in the specificity/degeneracy of peptide recognition. For the more specific clones amino acid identity was required at the primary TCR contact residues, whereas every TCR contact residue could be substituted by at least one structurally related amino acid for the more degenerate clones ([25,26]; M Martin, K Wucherpfennig, unpublished data). This illustrates the specificity/degeneracy distribution pattern proposed in Figure 1, in which the more specific T cells are represented in the left part of the main curve and the more degenerate ones on the right, with a small fraction of T cells at the extreme ends. An example of a highly degenerate T cell clone has recently been described [29•]. (The clone published by Hemmer et al. was specific for MBP(85–99). For most experiments, however, the authors used the MBP(88–98) peptide, which was activated by the randomized peptide libraries). This MBP(85–99)-specific T cell clone was stimulated by synthetic random peptide libraries in which every peptide position was randomized. These libraries consist of a large number of different peptides (2×10^{14} different sequences for an X11 library). Such a degree of degeneracy may not be very common as other MBP(85–99)-specific T cell clones were not stimulated by such random peptide libraries (M Martin, K Wucherpfennig, unpublished data). Notably, the T cell clones that cross-reacted with a diverse set of microbial peptide sequences were not stimulated by these random peptide libraries indicating that such a level of degeneracy in peptide recognition is not required for T cell cross-reactivity with microbial peptides (M Martin, K Wucherpfennig, unpublished data).

Another interesting aspect of the MBP(85–99) peptide is the fact that it also represents the immunodominant antibody epitope of MBP [34]. Moreover, the same core region of the peptide was important for both antibody binding and recognition by HLA-DR2 restricted T cell clones. Important differences, however, in the antibody and T cell epitopes were: firstly, a longer peptide was required for optimal T cell stimulation than for antibody binding, and secondly, sequence identity was required at every peptide position in the core of the antibody epitope whereas the MHC molecule contact residues in the T cell epitope could be substituted by other amino acids. Due to the similarity between the antibody and T-cell epitopes, a human papillomavirus peptide was recognized both by MBP-specific antibodies and by a MBP(85–99)-specific T cell clone [35•].

Taken together, the analysis of MBP-specific T cell clones from MS patients demonstrates that microbial peptides with limited sequence homology are effective activators of autoreactive T cells. These results make it unlikely that a single microbial peptide is responsible for the activation of autoreactive T cells in human autoimmune diseases. Other cross-reactive peptides of the MBP(85–99)

Table 1

Examples of experimentally determined T cell cross-reactivity between self and microbial peptides in human autoimmune diseases.

Disease, characteristics and reference	Organism/pathogen	Antigen	Sequence alignment*
Multiple sclerosis [3] CD4+ T cell clones HLA-DR2b- or HLA-DQ1 restricted	Human Herpes simplex virus Herpes simplex virus Epstein–Barr virus Adenovirus type 12 virus Influenza type A virus Pseudomonas aeruginosa Reovirus type 3 Human papillomavirus type 7	MBP (85–99) UL15 protein DNA polymerase DNA polymerase ORF Hemagglutinin Phosphomannomutase Sigma 2 protein L2 protein	**ENPVVHFFKNIVTPR** FRQLV**HF**VRDFAQLL GGRRLF**FVK**AHVRES TGGVY**HF**V**K**KHVHES DFEVV**TFLKD**VLPEF YRNLVW**F**I**KKN**TRYP DRLLML**FAKDVV**SRN MARAAFL**FK**TVGFGG IGGR**VHFFKD**ISPIA
Multiple sclerosis [29•] CD4+ T cell clone HLA-DR2b-restricted [29•]	Human Schizosaccharomyces pombe Human cytomegalovirus Salmonella typhimurium	MBP (88–98) Protein kinase CHK1 UL71 UDP-N-acetylenolpyruvoyl-glucosamine reductase	**VVHFFKNIVTP** WRK**FFKN**VVSS DILIL**KL**VVGE AGS**FFKN**PVVA
Insulin-dependent diabetes mellitus [53] Peripheral blood lymphocytes	Human Coxsackie virus	GAD (254–269) P2-C (32–47)	**ARFKMFPEVKEKGMAA** LKVKIL**PEVKEK**HEFL
Rheumatoid arthritis [54] CD4+ T cell clone	Human Mycobacterium bovis	hsp65 (267–281) hsp65 (241–255)	**HRKPLVIIAEDVDGE** AGKPLL**IIAEDV**EGE
Primary biliary cirrhosis [55] CD4+ T cell clone HLA-DR-restricted	Human Human Escherichia coli	PDC-E2(163–176) PDC-E2(36–49) PDC-E2(31–44)/(134–147)	**GDLLAEIETDKATI** **GDLIAEVETDKATV** EQSL**IT**V**E**G**DKA**SM

*Bold indicates amino acids that are identical with the self-peptide. GAD, glutamate decarboxylase; ORF, open reading frame; PDC, pyruvate dehydrogenase complex.

epitope remain to be identified as only parts of the microbial genome sequences are so far known and as only a fraction of candidate sequences have been synthesized and tested to date. Also, MBP-specific T cell lines from MS patients were found to cross-react with human coronavirus and other human pathogens; the cross-reactive peptide sequences await identification [36].

Cross-reactive T-cell epitopes have also been identified for autoantigens in other human autoimmune diseases (Table 1). While it is not yet known whether these autoantigens/microbial peptides are important in the development of these autoimmune diseases, these examples demonstrate that cross-reactive T cell epitopes can be identified.

Many studies have also examined the issue of cross-reactivity by merely performing sequence alignments between candidate self-antigens and microbial sequences. In the absence of functional data, these studies suffer from the following related problems: firstly, it is frequently not known whether the region of the autoantigen that was studied represents a T cell epitope; secondly, peptides that have visual sequence similarity may have no biological activity (e.g. lack of MHC molecule binding, failure to activate autoreactive T cells); and thirdly, peptides that are biologically active may be overlooked because the struc-

tural similarity to the self-peptide may not be obvious. The observations made in the analysis of MBP-specific T cell clones indicate that a detailed characterization of the immunodominant epitope of an autoantigen is required for the identification of cross-reactive microbial peptides.

Induction of experimental autoimmunity by microbial peptides that cross-react with self-antigens

Several animal models offer important insights into the induction of autoimmunity by infectious agents. Environmental exposure is important in the development of spontaneous autoimmunity in two transgenic animal models. Mice transgenic for a murine MBP (acetylated[Ac]1–11)-specific TCR develop spontaneous CNS autoimmunity under normal housing conditions but not in a specific pathogen-free environment [37,38•]. Likewise, HLA-B27 transgenic rats develop symptoms that resemble ankylosing spondylitis less frequently under germ-free conditions [35]. Both models indicate that exposure to microbial agents is important in the development of spontaneous autoimmunity. The microbial antigens/superantigens that may be responsible have not yet been defined.

The development of autoimmunity by microbial antigens has also been studied by immunization with microbial

Table 2

Examples of experimentally determined T cell cross-reactivity between self and microbial peptides in experimental animal models.

Experimental model and reference	Organism/pathogen	Antigen	Sequence alignment*
Experimental autoimmune uveitis [39]	Rat	Retinal S-antigen	**DTNLASSTIIKE**
Lewis rats immunized with foreign peptides	Yeast	Histone H3	**DTNLA**AIHAKRV
T-cell cross-reactivity	Escherichia coli	Hypothetical protein	LA**NLASST**QLCK
Disease induction	Hepatitis B virus	DNA polymerase	L**TNLL**S**S**NLSWL
	AKV murine leukemia virus	Gag-pol polyprotein	P**TNLA**KVKGITQ
Experimental ovarian autoimmune disease [40]	Mouse	Ovarian zona pellucida 3 peptide	**NSSSSQFQIHG**
B6AF₁ mice immunized with foreign peptides	Papilloma virus type Ia	L2 protein (486–494)	**NN**STGD**F**EL
Disease induction	Chlamydia psittacii	Outer membrane protein (224–234)	I**SS**PT**QF**VI**H**K
Experimental autoimmune encephalomyelitis [36]	Rabbit	MBP (66–75)	**TTHYGSLPNK**
New Zealand white rabbits immunized with hepatitis B virus peptide	Hepatitis B virus	Polymerase (589–598)	IGC**YGSLPN**E
Nonobese diabetic (NOD) mouse [56]	Mouse	GAD (254–269)	**ARYKMFPEVKEKGMAA**
NOD mice immunized with peptide activation of lymph node cells [56]	Coxsackie virus	P2-C (32–47)	LKVKIL**PEVKEK**HEFL
Adjuvant induced arthritis [57]	Mouse	hsp60 (162–170)	**KDIGNIISDA**
C57BL/6 mice immunized with mycobacterial hsp60	Mycobacterium bovis	hsp60 (499–508)	SAL**QN**AA**SI**A
T cell clone			
H-2Dᵇ-restricted			

*Bold indicates amino acids that are identical with the self-peptide. GAD, glutamate decarboxylase.

antigens/peptides that have sequence homology with immunodominant T-cell epitopes (Table 2). The first of these studies [36] examined the induction of experimental autoimmune encephalomyelitis (EAE) using a hepatitis B virus polymerase peptide that shared identity at six amino acid positions with the encephalitogenic site of rabbit MBP. Following immunization of rabbits with this peptide, antibody cross-reactivity and a certain degree of T cell cross-reactivity to MBP was observed. Four out of eleven animals showed histological signs of EAE [36]. Analysis of the amino-terminal MBP peptide (Ac1–11), which is immunodominant in B10.PL and PL/J mice, demonstrated that this epitope could be shortened to a seven amino acid peptide without the loss of encephalitogenicity. When tested with alanine analogs, identity at four amino acid positions was found to be required for T-cell cross-reactivity whereas five position were necessary for the induction of EAE [37,38]. The importance of the amino-terminal acetylation, however, which is absent in viral and bacterial peptides, may make it difficult to identify microbial peptides that induce EAE.

In two experimental models — experimental autoimmune uveitis and experimental ovarian autoimmune disease — microbial peptides have been identified that induce tissue specific autoimmunity. Experimental autoimmune uveitis in Lewis rats is mediated by T cells specific for the retinal S-antigen (reviewed in [39]). S-antigen specific autoimmunity could be induced by several microbial peptides which shared identity with five or six amino acids of the uveitogenic epitope of the S-antigen, although higher doses (10- to 40-fold) of the microbial peptides were

required. In experimental ovarian autoimmune disease, immunization with microbial peptides similar to the target antigen zona pellucida 3 protein induced an autoimmune oophoritis [44].

Tissue-specific autoimmunity has also been induced by immunization with whole microbial antigens. Lewis rats are highly sensitive to the development of arthritis following immunization with heat-killed *Mycobacterium tuberculosis* in mineral oil (complete Freund's adjuvant). A T cell response against the Mycobacterial heat shock protein (hsp) 65 appears to play an important role, as a T cell clone specific for the mycobacterial hsp65(180–188) peptide can transfer disease to naive, irradiated recipients (reviewed in [45]). The mycobacterial-specific T cell clone was found to cross-react with a proteoglycan found in joints; the peptide sequence of the cross-reactive self-epitope is not yet known. Whereas an hsp65(180–188)-specific T cell clone can transfer disease, neither the hsp peptide nor the hsp65 antigen are sufficient to induce arthritis, suggesting that other mycobacterial components that are pathogenic are necessary for inducing T cells to this epitope [46,47].

In the examples discussed above autoimmunity was induced by immunization with a peptide or a protein, which is artificial. The question of whether challenge with an infectious virus can initiate an autoimmune process by activating autoreactive cells was addressed by using transgenic mice that selectively express a viral antigen in the target organ (reviewed in [44]). Mice expressing the lymphocytic choriomeningitis viral glycoprotein or nucleoprotein in pancreatic β cells [45,46] or oligoden-

drocytes [47•] develop diabetes or CNS autoimmunity (although no EAE), respectively, after infection with the lymphocytic choriomeningitis virus. These studies show that viral infection can specifically break tolerance towards a 'self' antigen and initiate autoimmune disease. Further studies in these animal models [48–51] reveal that multiple other factors, for example expression of cytokines and costimulatory molecules, are of critical importance for disease development, which supports the notion that initiation of an autoimmune disease is a multistep/multifactorial process.

Requirements for the development of autoimmunity following activation of autoreactive T cells

Genome wide searches in human autoimmune diseases and in experimental animal models indicate that multiple genes (both MHC and non-MHC) are required for the development of spontaneous autoimmunity [17–19,52]. In susceptible individuals, the activation of autoreactive T cells is necessary but not sufficient for the development of an autoimmune disease (Table 3). An insufficient degree of clonal expansion of autoreactive T cells may be a major limiting factor. As some autoreactive T cell clones are activated by peptides from different pathogens a stepwise expansion of autoreactive T cells by subsequent infections is possible. Another limiting factor may be the functional phenotype induced by the infectious agent. In the EAE model, only a small fraction of MBP-specific T cell clones is pathogenic (<10%) and the presence of a Th1 phenotype alone is insufficient to induce EAE (S Brocke, personal communication). Therefore, autoreactive T cells may only be pathogenic if a particular functional program is induced by the infectious agent. Other factors that limit the occurrence of an autoimmune disease are access to the target organ, in addition to the expression levels of MHC class II and costimulatory molecules in the target organ. This is illustrated by the observation that in mice transgenic for an MBP(Ac1–11)-specific TCR, the *in vivo* activation of the autoreactive T cells, even with the native MBP peptide, is not sufficient to induce EAE unless the entry of those cells into the CNS is facilitated by pertussis toxin [34•].

Table 3

Requirements for the development of chronic autoimmune disease following activation of autoreactive T cells.

1. Sufficient clonal expansion of autoreactive T cells
2. Induction of a functional phenotype (i.e. cytokine profile) that makes T cells pathogenic
3. Entry of activated autoreactive T cells into the target organ
4. Sufficient expression levels of MHC molecules and costimulatory molecules on antigen presenting cells in the target organ

Conclusions

Activation of autoreactive T cells is important in the induction of human autoimmune diseases. Several studies

over the past two years have demonstrated that human T cell clones can recognize several peptide ligands that are quite distinct in their primary sequence. T cell clones that are more degenerate in the recognition of MHC molecule-bound peptides are more likely to cross-react with a self-antigen and to therefore induce autoimmunity.

Acknowledgements

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