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REVIEW ARTICLE

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Specificity, degeneracy, and molecular mimicry in antigen recognition by HLA-Class II restricted T cell receptors: implications for clinical medicine

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Abstract In humans, increased susceptibility to specific autoimmune diseases is closely associated with specific HLA-class II alleles. $CD4^+$ T cells that recognize short self-peptides in the context of HLA-class II molecules via their T cell receptor (TCR) are considered to mediate the central role of pathogenesis in autoimmunity. Although both self- and nonself-peptides are presented on HLA-class II molecules under physiological conditions, several mechanisms exist to avoid the T cell response to the self-peptide/HLA-class II complex. One of the mechanisms that account for the breakdown in immune tolerance is cross-recognition by TCR between a pathogen-derived antigen and a host antigen (molecular mimicry theory). Epidemiological studies have indicated that a number of autoimmune diseases are developed or exacerbated after infections. Therefore, elucidating the recognition nature of HLA-class II restricted TCR in detail is necessary in order to understand disease processes. A large body of evidence indicates that T cell recognition is highly degenerate, and many different peptides can activate an individual T cell. Degeneracy of TCR recognition also can appear in various physiological outcomes, ranging from full activation to strong antagonism. Here, we review the clinical implications of our findings on T cell recognition, as well as a new direction of future applications for analyses in molecular mimicry. We also describe the latest developments in methods of mapping TCR epitopes for $CD4^+$ T cells using a peptide epitope expression library generated in the class II-associated invariant chain peptide substituted invariant chain gene format.

Key words Autoimmunity · Epitopes · HLA · Molecular mimicry · T cell receptors (TCR)

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Introduction

Particular class-II human histocompatibility leukocyte antigen (HLA-class II) alleles are associated with susceptibility to particular autoimmune diseases.[1] $CD4^+$ T cells recognize 10–20 amino acid long peptides in the context of class II molecules expressed on antigen-presenting cells through their T cell receptor (TCR). Autoreactive $CD4^+$ T cells are considered to have a central role in development of autoimmune diseases. Even in the presence of exogenous nonself antigens, the majority of HLA-class II molecules bind self-peptides processed mainly from self-membrane or secretory proteins. If the density of self-peptides/HLA-class II complexes expressed on the surface of cells is large enough to ensure a high avidity engagement of TCR, most autoreactive $CD4^+$ T cells are deleted in the thymus or become anergic in the periphery. If the density of self-peptide/HLA-class II complexes is small enough not to activate T cells in the periphery, T cells do not need to acquire tolerance to such complexes and ignore them.[2] Thereby, $CD4^+$ T cells do not respond to these self-peptides in the context of self HLA-class II molecules, except in autoimmune states.

Epidemiological studies have indicated that a number of autoimmune diseases either develop or are exacerbated after infections. Several possible mechanisms can account

for the clinical association between microbial infection and the clonal expansion of autoreactive T cells as a cause of autoimmune disease. One mechanism is the activation and expansion of autoreactive T cells by an antigen nonspecific inflammatory stimulus of the innate immune system.[3] Microorganism-induced activation of antigen-presenting cells (APCs) leads to the release of inflammatory cytokines and/or chemokines, thus enhancing antigen-processing, and the up-regulation of peptide–MHC complexes and co-stimulatory molecules on the cell surface (microbial adjuvant effects).[4–8] Local infection causes tissue destruction, and the release of sequestered antigen follows.[9,10] The inflammatory state can also promote the expansion of memory T cell populations (bystander activation).[3,11] Another mechanism is that microbial superantigens activate large numbers of T cells expressing particular Vβ gene segments, and a limited population can cross-react to a self-antigen.[12–14] Cross-recognition by T cells between self-antigens and infectious agents is another important mechanism (molecular mimicry theory).[15,16] In any case, tissue destruction would be expanded by epitope spreading. These concepts are useful to explain the as yet unsolved mechanisms for the etiological linkage between infection and autoimmunity.

The concept of molecular mimicry was broadened by recent insights into T cell recognition. Molecular mimicry phenomena were noted in disease-associated autoreactive $CD4^+$ T cells in autoimmune disorders such as multiple sclerosis (MS), type I diabetes, and Lyme arthritis in humans.[17–20] Lyme disease, a chronic inflammatory joint disease, is caused by infection with the spirochete *Borrelia burgdorferi*.[19] Susceptibility to Lyme arthritis is associated with HLA-DR4 and HLA-DR1 alleles. It has been reported that synovial fluid T cells from patients with treatment-resistant Lyme disease showed a strong response to outer surface proteins A (OspA) of *B. burgdorferi* and cross-reacted to self LFA-1. The sequence homology between OspA p165–173 and the αL-chain of hLFA-1 p332–340 was considered to be a basis for molecular mimicry in treatment-resistant Lyme disease.[19] On the other hand, the association between microbial infection and autoimmune disease has also been investigated using a mouse model.[21–24] In a model of herpes simplex virus (HSV) type-I induced herpes stromal keratitis (HSK), which is a T cell-mediated inflammatory disease of the cornea, corneal antigen autoreactive T cells recognized HSV-1 UL-6 protein.[21] However, an HSV-1 point mutant that contains a single amino acid substitution within the putative mimicry epitope impaired its capacity to induce HSK.[22] In this system, two different pathogenic pathways, innate immune mechanisms and molecular mimicry, are involved. In this report, mimicry was considered to be essential for disease induction with a limited number of autoreactive T cells, while innate immune mechanisms are also important to provoke disease with high numbers of autoreactive T cells.

Because of the importance of $CD4^+$ T cells in the development of autoimmunity, efforts have been directed toward the identification of cross-reactive epitopes of microbial antigens recognized by autoreactive $CD4^+$ T cells. For many years, antigen recognition by TCR was considered to be highly specific, and the concept of molecular mimicry had been defined based on the level of primary sequence similarities between self and antigenic determinants of infectious microorganisms.[25] Since the 1990s, studies using peptide analogues with single amino acid substitution or positional scanning synthetic combinatorial peptide libraries (PS-SCLs) have demonstrated that antigen recognition by TCR is highly degenerate and many different peptides can activate an individual T cell.[26–31] Wucherpfennig and Strominger[27] reported that microbial peptides with a relatively limited sequence homology to myelin basic protein (MBP) could activate MBP autoreactive T cell clones. Using PS-SCLs, Hemmer et al.[28] noted differing recognition profiles in individual autoreactive T cell clones from patients with MS, and predicted stimulatory ligands that showed no sequence homology with the known cognate peptide. Therefore, molecular mimicry may be a more frequent event than was generally assumed.

Here, we focus on: (1) degeneracy in antigen recognition by TCR; (2) differences in the physiological outcomes of T cell responses manipulated by altered peptide ligands; (3) the latest methods for the identification of diverse TCR epitopes for $CD4^+$ T cell clones.

Structure of the TCR-peptide/HLA-class II complex

The HLA-class II molecules are heterodimeric membrane glycoproteins consisting of α and β chains. DRα chains are monomorphic, but DPα and DQα chains and β chains are highly polymorphic. The molecule has a peptide-binding groove on the top of the molecule and binds antigenic peptides processed by APCs such as dendritic cells (DCs) or B cells. The structural requirements for HLA-class II binding peptides have been analyzed in detail, and peptide binding motifs specific for various human and mouse class II molecules have been reported.[32] Three to five amino acid residues, separated from each other by one to two intervening residue(s), acted as anchor residue(s), for binding to HLA-class II molecules.[33] On the other hand, side chains of amino acid residues flanking anchor residues were the main recognition sites for TCR. This view was clearly established in crystallographic analyses of the DR molecules bound by either self-[34] or nonself-peptides.[35] Sixty-five percent of the peptide surface made contact with the DR molecule, and the remaining portion was accessible to solvents, thus being recognized by the TCR. Most pockets in the groove of the HLA-class II molecules are shaped by clusters of polymorphic residues, indicating that the class II allelic variant has a major effect on differences in the structures of bound peptides, and determines the individual differences in T cell responses to a given antigenic peptide.

TCR is composed of two membrane-anchored polypeptides, α and β chains, and each chain consists of one constant (C) and one variable (V) domain. The TCR Vα or Vβ regions are composed of V-Jα or V-D-Jβ productively rear-

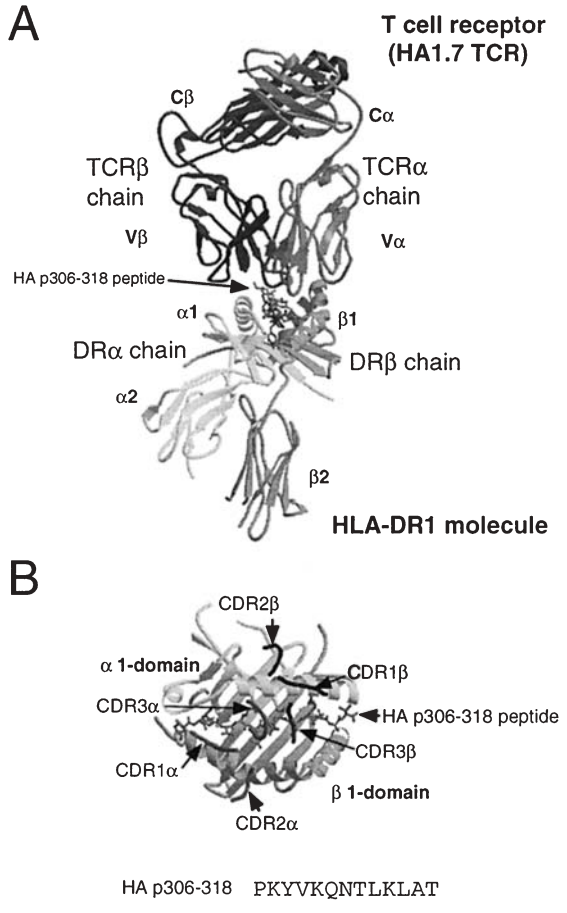

Fig. 1. **A** Structure of the HA1.7 TCR–HA/DR1 complex[40] with TCR at the top and DR1 at the bottom. The T cell clone HA1.7 is specific to HA p306–318 in the context of HLA-DR1. α1, α2, β1, and β2 indicate the extracellular domains of α and β chains of HLA class II molecules. Vα, Vβ, Cα, and Cβ indicate the variable and constant regions of T cell receptor α or β chains, respectively. **B** The structure of HA p306–318/HLA-DR1 complex and relative orientation of the CDR loops of HA1.7 TCR on top of HA/DR1 complex. CDR loops of TCR Vα and Vβ chains are displayed in tubes. HA p306–318 peptide is shown in a ball-and-stick model. The top of α1 and β1 domains create a groove-like structure consisting of a β-sheet floor and two side walls made of two antiparallel α-helices. The peptide sequences of HA p306–308 are given in a single-letter amino acid code. This figure was produced by BOBSCRIPT[41]

ranged gene products.[36] The complementary determining regions (CDRs) are hypervariable loops at one end of the TCR that recognize the HLA molecule and the antigenic surface derived from the solvent-accessible side chains of amino acid residues flanking HLA anchoring residues.

Recent crystallographic studies of TCR–peptide/MHC complexes provide a structural basis for antigen recognition by αβTCRs.[37] All αβTCRs represent a relatively flat surface bound to the peptide/MHC complex, and represent a similar binding mode. The angle between the peptide direction and the long axis of the class I-restricted TCR interface is between 45 and 70° (diagonal mode).[38] In contrast, the class II-restricted TCR interface is between 70 and 80° (orthogonal mode).[39,40] Figure 1 shows a recent model of the crystallographic structure of the HA1.7 TCR-HA p306–318/DR1 complex. The CDR1 loops of the TCR V-regions contact both peptide and MHC molecules. On the other hand, the CDR2 loops contact prominent α-helices of the MHC molecule. The long CDR3 loops of the TCR V-regions extend down over the center of the antigenic peptide. TCR contacts span only nine residues (P − 1–P8) of the antigenic peptide in both human and murine TCR-peptide/MHC II complexes.

Differences in physiological outcomes of T cell responses manipulated by altered peptide ligands

Altered peptide ligands (APLs) represent a useful tool for studying differential recognition by TCR. It was previously considered that the recognition and response of T cells were apparently an on/off phenomenon. However, findings in mice utilizing peptide analogues with a single residue substitution revealed that T cell clones recognize these APLs and altered T cell responses occur. APLs induced T cell nonresponsiveness through TCR antagonism[42,43] or the induction of anergy as a consequence of partial activation,[44,45] and sometimes induced dissociation between proliferative response and cytokine production.[46,47] Some peptide analogues with antagonistic properties for TCR partially stimulated T cells to induce increases in cell size and expression levels of CD11a (LFA-1) and CD25 (IL-2R) on the T cell surface, but not proliferation.[44] Analyses of the physical interactions of purified TCR with MHC–APL complexes revealed differences in the half-life of receptor–ligand interactions. These phenomena led to differences in the signals transduced by the TCR, resulting in differences in functional outcomes.[48]

We analyzed the responses of human $CD4^+$ T cell clones to a large number of peptide analogues.[29,31] Figure 2 shows a summary of the antagonistic activity of APLs in an acetylcholine receptor (AChR) α subunit autoreactive T cell clone SK2.11, established from a patient with infant-onset myasthenia gravis (MG).[31] Infant-onset MG is unique to Asian populations, and disease susceptibility is strongly associated with DR9 (DRB1*0901)-DQ9 (DQA1*0301-DQB1*0303) and DR13 (DRB1*1302)-DQ6 (DQA1*0102-DQB1*0604) haplotypes that are also unique to Asians. This T cell clone recognizes AchRα p71–91 in the context of disease-susceptible DQ6 (DQA1*0102-DQB1*0604). Although the majority of analogues substituted at residues Phe-77, Leu-80, and Asn-82 stimulated proliferation of the T cell clone (data not shown), the majority of peptide analogues substituted at either Gln-81 or Glu-83, while those which were the most likely TCR contact residues showed antagonistic activity.

Figure 3 shows a summary of the responses of T cell clone YN5–32 specifically recognizing a streptococcal M12 p54–68 in the context of DR4 (DRB1*0406) to 156 independent peptide analogues with a single residue substitution. P1 (position 1) means the putative most N-terminal DR anchor residue.[29] The residues Leu-57 (P1), Ala-60 (P4), and Asn-62 (P6) were the most likely to be DR-anchor

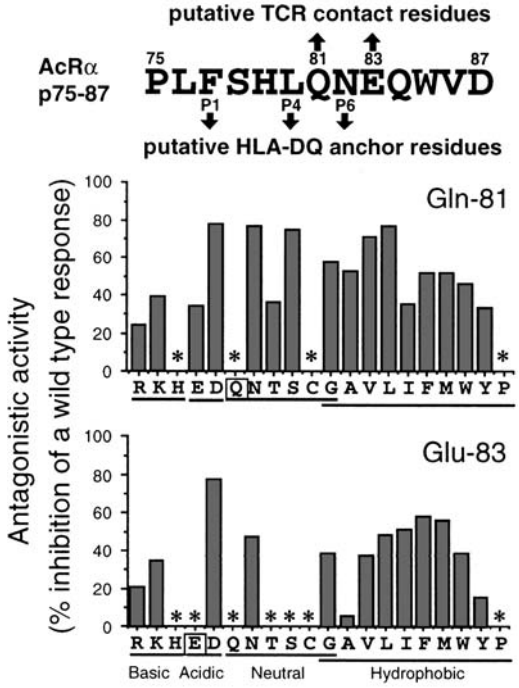

Fig. 2. Summary of antagonistic activities of peptide analogues with single-residue substitutions in human AchRα p75–87 peptide. T cell clone SK2.11 autoreactive to AchRα p75–87 in the context of HLA-DQ6 and established from a patient with infant-onset MG was cultured with irradiated APCs prepulsed with a minimal dose of AchRα p75–87 peptide in the presence of a large number of soluble peptide analogues. P1 (position 1) means the putative most N-terminal anchor residue. Antagonistic activity was expressed by the percentage inhibitory effect of the analogue on proliferative responses of SK2.11 to the wild-type peptide. The response was in the range 7000–10 000 cpm. *Asterisks* indicates not tested, because some of these analogues exhibited TCR agonism and because other analogues were not synthesized

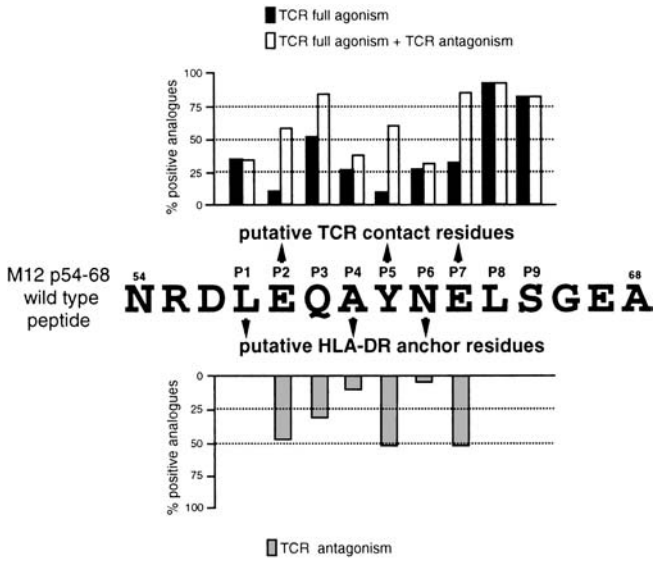

Fig. 3. Summary of responses of the HLA-DR4-restricted T cell clone YN5–32 to 156 peptide analogues with single-residue substitutions in a streptococcal M12 p54–68 peptide. From P1–P7 positions, residues were replaced with all other amino acides. Percentages of peptide analogues exhibiting either agonism or TCR antagonism are indicated for positions. Full agonism means stimulation of the proliferative responses of the T cell clone to various degrees. TCR antagonism means that an excess amount of peptide analogues inhibited proliferative responses of the T cell clone to a minimal dose of wild-type peptide, and without inducing T cell anergy. *Black bars*, TCR full agonism; *white bars*, TCR full agonism + TCR antagonism; *gray bars*, TCR antagonism

residues, and 30% (17/57) of peptide analogues substituted at these residues exhibited full agonism to stimulate various magnitudes of proliferative responses in the T cell clone. Only 7.5% (3/40) of not fully agonistic peptides exhibited TCR antagonism. On the other hand, residues Glu-58 (P2), Tyr-61 (P5), and Glu-63 (P7) were the most likely to be TCR-recognition sites, and only 15.8% (9/57) of analogues stimulated proliferative responses in YN5–32, which means that substitutions at these residues frequently abrogate T cell recognition.

Interestingly, as many as 60.4% (29/48) of analogues without fully agonistic properties exhibited TCR antagonism, which inhibited the proliferative response of YN5-32 to the wild-type peptide. Eight (27.6%) of these antagonistic analogues with relatively conservative amino acid substitution exhibited partial agonism, which induced increases in cell size and expression levels of CD4, CD11a (LFA-1), CD28, CD49d (VLA-4), and CD95 (Fas), and small increases in CD25 and CD44 expression on the T cell surface, as compared with responses to the wild-type peptide. The wild-type peptide (but not the partially agonistic APLs) induced down-modulation of CD3 expression and up-regulation of CD54 and CD69 expressions. None of the APLs with partially agonistic properties stimulated IFN-γ production and induced anergy.

These observations collectively indicate that: (1) different physiological outcomes are induced in the recognition of APLs in human $CD4^+$ T cell clones, as noted by other studies of murine T cells; (2) APLs with antagonistic properties were mainly observed by substitution at TCR contact sites, but substitution at the HLA anchoring residues also could contribute to TCR antagonism; (3) many APLs with substitution at HLA anchoring residues exhibit agonistic properties.

A slight alteration in an antigenic peptide or DR molecule, even far from the recognition surface, significantly affects recognition by TCR

Because single amino acid polymorphism at residue 37 of the HLA–DRβ chain (DRβ37) between DRB1*0406 and 0403 markedly influences susceptibility to the insulin autoimmune syndrome,[49] we investigated the effects of DRβ37 polymorphism on the recognition of nonself peptides by YN5-32.[50] As described above, 154 peptide analogues were tested for agonist and TCR antagonist properties. Of these, 46 analogues showed full agonism, 34 analogues exhibited TCR antagonism, and 45 analogues exhibited neither full agonism nor TCR antagonism, irrespective of the presenting molecules DRB1*0406 or

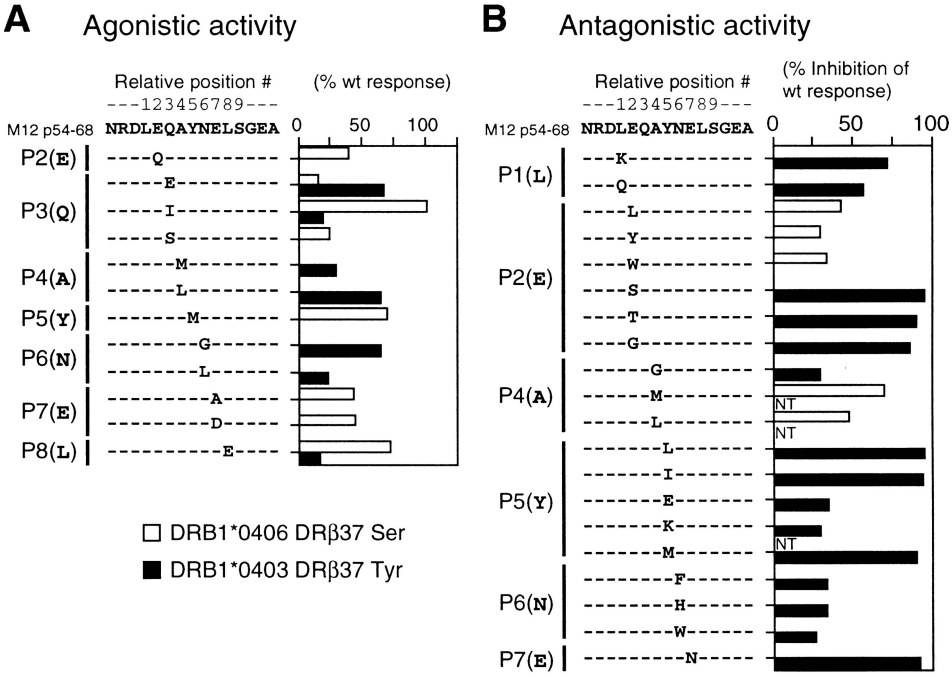

Fig. 4. Summary of distinct responses to peptide analogues of the T cell clone YN5-32 induced by DRβ37 single-residue polymorphism. Data are given as percentage wild-type response in peptide analogues exhibiting agonism, or percentage inhibition of wild-type response in peptide analogues exhibiting TCR antagonism. *Open* and *closed bars* indicate responses observed in presentations by DRB1*0406 and DRB1*0403, respectively. **A** Agonistic proliferative responses of YN5-32. **B** Antagonistic inhibition of proliferative responses of YN5-32

DRB1*0403. On the other hand, 29 analogues substituted at each of residues 57(P1)–63(P7) of M12p54–68 were recognized differently by YN5-32, depending on the presenting HLA–DR molecules. Figure 4 shows a summary of the distinct responses observed to 29 peptide analogues of YN5-32 induced by DRβ37 polymorphism. The agonistic and antagonistic activities of all the 29 analogues with a single substitution spanning the core epitope were clearly diverse. These observations indicate that single amino acid polymorphism (Ser–Tyr) at the DRβ37 residue induced conformational changes in peptides, which can be distinguished by TCR. This can be inferred from the differences in affinity between two DR4 molecules and peptides or between TCR and their ligands. These conformational changes were observed even in APLs with single residue substitutions at residues far from a putative DRβ37 contact site.

In recent studies, Kersh et al.[51,52] showed that TCRs can discriminate between two APLs in which only a single I-E^k P6 anchor residue was substituted for a chemically conservative one, which does not significantly alter the binding affinity to MHC. Alterations in the main chain conformation in P6–P8 and a slight change in the angle of the P8 TCR-contacted side chain were evident in a crystal analysis. As a result, the physiological response changed from full agonism to antagonism. This observation indicates that recognition by TCR is significantly affected by slight alterations far from the TCR recognition surface, and its physiological reactivity can be markedly changed.

Degeneracy in antigen recognition by TCR is not predictable by an independent contribution model

Much epidemiological evidence related to human autoimmune diseases is almost compatible with the molecular mimicry hypothesis for the development of disease. Indeed, subjects have been seen to develop autoimmune disease after infection. Elucidation of the structural requirements for peptides to be cross-recognized by autoreactive T cells is of great importance in understanding disease processes. To date, mimicry epitopes have been predicted and identified based on primary sequence homology, the data being obtained from single-residue substituted peptide analogues, or PS–SCLs.[53–55] PS–SCLs using synthetic peptide are the main means currently used, and are fundamentally based on the concept that the antigen recognition surface of TCR is relatively flat, and each amino acid in each position on the peptide independently contributes to recognition by TCR (independent contribution model).[56] In such systems, putative mimicry peptides were searched using algorithms designed by combining the amino acids selected for each position by referring to tested data. However, the combinations of amino acids used in these systems did not always function as expected. In fact, artificial peptides composed of the optimal residue for each position selected, and based on analysis with PS–SCLs, do not necessarily show agonistic activity.[57]

Although TCR shows a high degree of degeneracy in recognized peptides, a slight alteration of an antigenic peptide or a DR molecule, even far from the recognition surface, can affect TCRs, which show exquisite specificity. It has been reported that certain TCR recognition is affected by each amino acid adjacent or not adjacent to TCR contact residues, or by each amino acid combination in an antigenic

peptide.[58,59] Our extensive analysis of the combinatorial nature of epitopes recognized by TCRs, using a class II-associated invariant chain peptide (CLIP)-substituted epitope expression library, also indicated that recognition by TCR was significantly affected by combinations of amino acids in the antigenic peptide.[60] Therefore, the potency of a peptide to stimulate certain T cells cannot be predicted precisely in approaches based on an independent contribution model.

The use of an epitope expression library for systematic analysis of diverse cross-reactive epitopes

On the basis of these observations, we developed a new strategy to identify epitopes of HLA-class II restricted TCRs from a library of randomized 13-mer peptides.[61] The establishment of an efficient system for the delivery of antigenic peptides to the HLA-class II-restricted antigen presenting pathway should be useful for the development of a library of cells expressing a diverse array of peptides in the context of HLA-class II molecules. We prepared an oligonucleotide library by replacing a gene segment encoding for CLIP (invariant chain p89–101) of invariant chain (Ii) with double-stranded DNAs of randomized sequences, and prepared an epitope-presenting library which loads randomized peptides onto HLA-class II molecules co-expressed in COS-7 cells. The use of an invariant chain with targeting signals to endosomes is a pertinent strategy for antigen presentation to $CD4^+$ T cells.[62–65] This approach, by which multiple residues were simultaneously randomized, has the great advantage of producing overall conformation of the peptide/HLA-class II complexes, and increasing the possibility of finding degenerate sequences with agonistic properties.

The screening system is depicted schematically in Fig. 5. Plasmid clones encoding epitopes agonistic to $CD4^+$ T cells can be identified by measuring the IFN-γ produced by the stimulated T cells. The sensitivity of the screening was considered to be sufficient to identify unknown epitopes in the presence of more than 1000 irrelevant clones in a single well of a 96-well plate, and it is possible to screen 10^5 clones using one 96-well plate (Fig. 6). As a model system, we searched for a cross-reactive epitope for a T cell clone SA32.5 specific to glutamic acid decarboxylase (GAD) 65, an autoantigen implicated in type I diabetes.[66] Screening a library containing 2×10^5 independent plasmid clones isolated a plasmid clone, designated pCIGm1, that stimulates SA 32.5 (Fig. 7A). We investigated whether the epitope encoded by the inserted DNA in pCIGm1 was stimulatory for SA 32.5 when added as a synthetic peptide. We synthesized four peptides (Gm1.1–1.4) containing all or part of the 13-mer peptide (QLSNQWHVVGATF) substituted for CLIP (Ii p89–101), together with different flanking sequences derived from Ii, and examined the capacity to stimulate SA 32.5. The T cell stimulatory activity of these peptides was tested by proliferation assay in which the T cell clone and DR53-positive irradiated peripheral

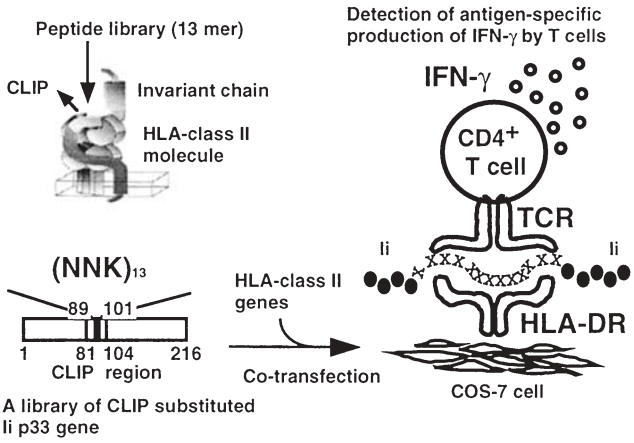

Fig. 5. The expression cloning system to identify epitopes for $CD4^+$ T cells using the CLIP-substituted Ii-chain-based epitope presenting library. The screening system to identify epitopes from the epitope library and construct the epitope library using the CLIP-substituted Ii p33 gene are presented schematically. Plasmid DNA of the epitope library is introduced along with HLA class II expression vectors into COS-7 cells. Transiently transfected COS-7 cells, which express a diverse array of peptides in the context of class II molecules, are co-cultured with $CD4^+$ T cells in 96-well culture plates. The response of stimulated T cells is detected by the production of IFN-γ if agonistic epitopes are expressed in the well. DNA sequencing of CLIP inserts determined the agonistic epitope sequences. *N* means any nucleotide, *K* means guanin (G) or (thymine) T, and *X* means any amino acid

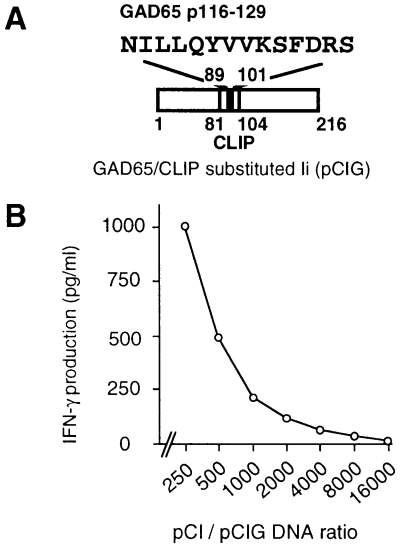

Fig. 6. Determination of the sensitivity of screening for T cell epitopes using the pCI expression vector. **A** Construct of pCIG, the GAD65-epitope-presenting vector in which human GAD65 p116–129 was inserted to pCI. **B** pCIG as relevant stimulative plasmid DNA was diluted with pCI as irrelevant plasmid at graded ratios. The mixtures of plasmids (pCI/pCIG) along with HLA-DRA*0101 and HLA-DRB4*0103 genes were transfected into COS-7 cells in a well of the 96-well plate. After overnight culture, GAD65 p116–129 specific T cell clone SA 32.5 (5×10^4 cells/well) were added to COS-7 cells followed by 48 h culture. The amount of IFN-γ in the supernatants was measured using ELISA. Results are expressed as means of triplicate determinations ± SD. *Circles* indicate IFN-γ produced in a well in which COS-7 cells were transfected with the pCI, pCIG, and DR expression vectors

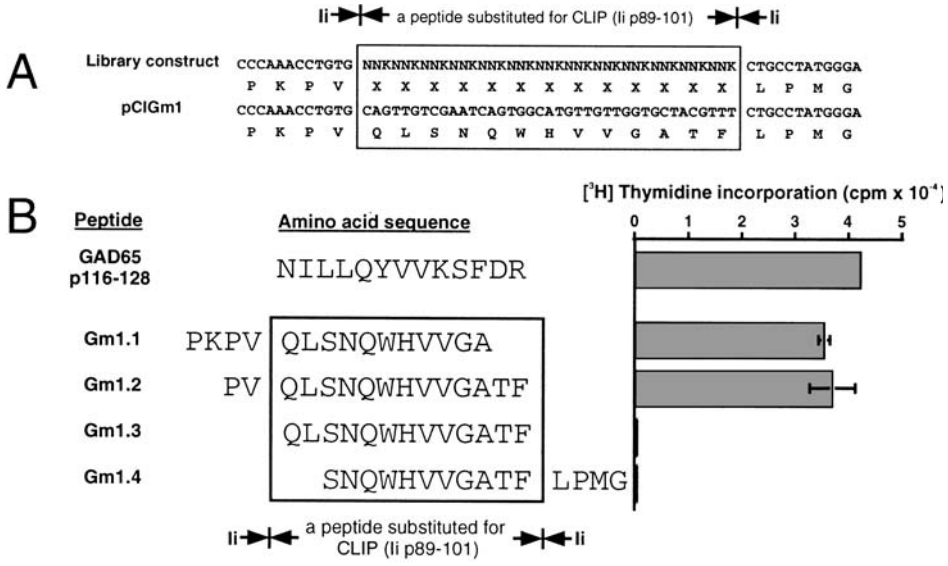

Fig. 7. Identification of a cross-reactive epitope for a GAD-65-specific T cell clone SA 32.5. **A** The construct of a CLIP-substituted peptide library and the peptide encoded by the insert DNA of the plasmid clone pCIGm1 identified by screening of the library. *X* indicates a random amino acid encoded by nucleotide triplets NNK, where *N* stands for an equal mixture of the deoxynucleotides G, A, T, and C, and *K* stands for an equal mixture of G and T. **B** Determination of a cross-reactive epitope using synthetic peptides. The amino acid sequence encoded by the nucleotide sequence, substituted for CLIP of the isolated plasmid clone pCIGm1 was QLSNQWHVVGATF. Four overlapping peptides containing all or part of this sequence, together with Ii-derived flanking sequences, were synthesized. To investigate the T cell stimulatory activities of these peptides, a T cell proliferation assay was carried out. The T cell clone SA 32.5 (3×10^4 cells/well) was cultured in the presence of 50-μM tested peptides and irradiated HLA-DR53-positive PBMC (1.5×10^5 cells/well) for 72 h in wells of a 96-well plate, and the proliferative responses were measured by counting [^{3}H]-TdR incorporation. Results are expressed as means of triplicate determinations ± SD

blood mononuclear cells (PBMC) were co-cultured in the presence of 50 μM peptide (Fig. 7B). Of the four peptides, Gm1.1 (PKPVQLSNQWHVVGA) and Gm1.2 (PVQLSNQWHVVGATF), containing all or part of the insert sequence with two or four CLIP-flanking residues at the N-terminus, stimulated SA 32.5, but peptide Gm1.3, with a deletion of two amino acids at the N-terminus of the 13-mer insert, did not do so. Also, the proliferative responses of SA 32.5 to both peptides increased in a peptide dose-dependent manner (data not shown), and the Gm1.2 peptide stimulated comparable responses to those induced by the GAD65 p116–128. Although, the newly identified epitope (PVQLSNQWHVVGATF) was very different from the original epitope, GAD65 p116–128 (NILLQYVVKSFDR), it did have a capacity to stimulate the T cell clone which was comparable to that seen with the original GAD epitope.

Structural mimicry for the future direction of research on molecular mimicry

In the past few years, it has been considered that the application of APLs to down-regulate the responses of pathogenic T cells would be of therapeutic value in treating subjects with autoimmune disease.[67–70] Some TCR antagonists can induce anergy and change the cytokine profiles of T cell clones.[69,70] The secretion of anti-inflammatory cytokines IL-4, IL-10, and TGF-β by autoreactive T cells is associated with potent bystander suppression. APLs allow the in vivo generation of IL-4- IL-10- and TGF-β-secreting autoreactive T cells capable of migrating to the autoimmune site and suppressing local inflammatory responses. The injection of APLs is of clear therapeutic value in treating models of experimental encephalomyelitis (EAE).[68] In this case, the application of APL selectively silences pathogenic T cells, and other nonpathogenic T cells are recruited to the site of the disease as a result of the production of IL-4 and the reduction of TNF-α in the lesion.

However, it is difficult to find APLs of self-antigenic peptides that have no risk of activating autoreactive T cells. Recent clinical trials with an APL of MBP p85–99 for MS patients indicate that a high dose of an APL induced MBP-reactive T cells at a high frequency, resulting in the exacerbation of MRI-detectable lesions. In contrast, a low dose of an APL led to an immune deviation toward increases in IL-4 secretion by MBP-reactive T cells, with no progression in lesions.[71,72] This observation suggests that the engagement of diverse TCRs with a single APL derived from a self-antigen yields a variety of substantial cross-reactivities to a self-antigen. Although monoclonal or oligoclonal expansion of autoreactive T cells has proven to play a central role in the pathogenesis of autoimmunity, T cell populations which recognize certain self-antigens are relatively more diverse. Thus, a completely different sequence that mimics self-antigen may be a key epitope that activates a limited fraction of autoreactive T cells. Our findings indicate that

the cross-reactivity of TCR can be triggered by the peptide with, unexpectedly, no resemblance to the epitope of the autoantigen. Therefore, it may be more effective to use TCR antagonists of microbial mimicry epitopes that down-regulate the limited fraction of self-reactive T cells.

Recently, the mode of recognition by a single autoreactive TCR of two independent peptides in the context of two different DR2 molecules was structurally presented.[73] The T cell clone established from an MS patient cross-recognized MBP p85–99 in the context of DRB1*1501 and EBV DNA polymerase p627–641 peptide in the context of DRB5*0101. Both DRB alleles are in a strong linkage disequilibrium, and are associated with susceptibility to MS. This finding is not only important for molecular mimicry involving antigenic peptides, but also supports the structural basis of molecular mimicry generated by peptide–HLA-class II interactions.

Conclusions

Recent elucidation of the three-dimensional structures of the TCR-peptide/HLA-DR1 complex provides a structural basis for antigen recognition by HLA-class II restricted TCR. Flexibility in recognition by MHC-class I restricted TCR could also be structurally explained by large conformational changes of three CDR loops on binding to the ligand.[74] Similar changes in CDR loops of MHC-class II restricted TCR on binding can also be expected. The use of synthetic peptides in analyses of TCR recognition has provided a large number of useful concepts in T cell immunity. Using a set of peptide analogues, it has become clear that many modifications of the antigenic peptide are tolerated. Using extensive PS–SCLs, a high degree of degeneracy in TCR recognition has become evident. Although degeneracy in antigen recognition by TCR becomes an effective protection against infection in a limited T cell repertoire, and also helps maintain an immune system which can cope with a continuously changing antigenic environment, it may eventually cause autoimmunity through molecular mimicry. Previously, conformational changes in peptides on the groove of MHC class II molecules caused by binding could not have been predicted. This was also the case even if PS-SCLs were adapted. We have clearly shown that our epitope expression library system using CLIP-substituted Ii-genes provides an entirely new strategy not only for the identification of the cross-reactive epitopes for $CD4^+$ T cells of known specificity, but also for the detection of epitopes which stimulate $CD4^+$ T cells, the epitopes of which are unknown. Importantly, in analyzing molecular mimicry, this system deciphers overall conformations within epitopes on TCR recognition. The system also gives the prospect of providing a greater understanding of recognition mode in HLA-class II restricted TCR that could lead to preventive and therapeutic approaches.

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