Molecular analyses of HLA class II-associated susceptibility to subtypes of autoimmune diseases unique to Asians

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Abstract

It is well known that individuals positive for particular HLA-class II alleles show high risks for the development of Takayasu arteritis and other diseases caused by immunological disorders such as autoimmune diseases and allergies. HLA class II molecules present antigenic peptides to CD4+ T cells. Their extensive polymorphism affects the structures of peptides bound to HLA class II molecules to create individual differences in immune responses to antigenic peptides. To better understand the mechanisms for association between HLA class II alleles and susceptibility to autoimmune diseases, it is important to identify self-peptides presented by disease-susceptible HLA class II molecules and triggering disease-causative T cells. Many autoimmune diseases are observed in all ethnic groups, whereas the incidences of diseases, clinical manifestations and disease-susceptible HLA class II alleles are different among various ethnic groups for some autoimmune diseases. These phenomena suggest that differences in autoimmune self-peptide(s) in the context of disease-susceptible HLA class II molecules may cause these differences. Therefore, comparisons among disease-susceptible HLA class II alleles, autoimmune self-peptides and clinical manifestations of autoimmune diseases in different ethnic groups would be helpful in determining the pathogenesis of the diseases. In this paper, we describe our recent findings on: (1) the uniqueness of both clinical manifestations and HLA-linked genetic background of Asian-type (opticospinal form) multiple sclerosis; (2) the structural characteristics of peptides bound to HLA-DQ molecules susceptible to insulin-dependent diabetes mellitus; (3) the identification of a disease-related autoantigenic peptide presented by disease-susceptible HLA-DQ molecules in Asians-specific infant onset myasthenia gravis; and (4) a manipulation of human T cell response by altered peptide ligands, as a possible candidate for new and antigen-specific immuno-suppressive therapy against autoimmune diseases. © 1998 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: HLA class II molecule; Disease-susceptibility; Binding-peptide motifs; Autoimmune disease; Self-antigenic peptide; Autoreactive T cell

1. Introduction

The human leukocyte antigen class-II (HLA-II) molecule is a highly polymorphic heterodimeric membrane protein consisted of α and β chains and is expressed on B cells, antigen presenting cells (APC) and activated T cells. As shown in Fig. 1A, the HLA-II molecule has a peptide binding groove on top of the molecule and binds antigenic peptides processed by APC to present them to CD4+ T cells (see review in [1]). Even in the presence of exogenous non-self-antigens, the majority of HLA-II molecules bind self-peptides processed mainly from self-membrane or secretory proteins. If the density of HLA-II plus self-peptides is large, the majority of CD4+ T cells autoreactive to them is deleted in the thymus or energized in the periphery. If the density of HLA-II
peptides were determined in many combinations of HLA-II molecules and peptides [2–6]. They revealed that 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-II molecules. On the other hand, side chains of amino acid residues flanking anchor residues were the main recognition sites for the T cell receptor (TCR). This view was clearly established by crystallographic analyses of the DR molecules complexed with either self- [7–9] or non-self-peptides [10]. At least five DR anchor residues on the DR1 binding peptide and five corresponding independent pockets in the peptide-binding groove of DR1 molecule which accommodate side chains of DR anchor residues of the peptide were identified (Fig. 1B). 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. Many polymorphic residues of HLA-II molecules locate in the peptide-binding groove and were bound towards peptides. Thereby, polymorphism of HLA-II molecules determines differences in the structures of peptides bound to HLA-II molecules. In other words, polymorphism of HLA-II molecules determines individual differences in T cell response to given antigenic peptides.

The frequencies of particular HLA-II allele(s) or haplotype(s) are increased in patients with several diseases (see reviews in [11,12]), including Takayasu arteritis [13], rheumatoid arthritis (RA), insulin-dependent diabetes mellitus (IDDM), myasthenia gravis (MG), multiple sclerosis (MS) and insulin autoimmune syndrome (IAS or Hirata’s disease) [14] as compared with those observed in healthy controls. It is also well known that disease-associated HLA-II genes differ among different ethnic groups in some diseases [15]. For example, in the Japanese population, susceptibility to Takayasu arteritis is strongly associated with a HLA-B52-DR2(DRB1*1502-DRB5*0102)-DQ6(DQA1*0103-DQB1*0601)- DP9(DPA1*02-DPB1*0901) haplotype [13], whereas other HLA alleles are associated with the disease in Korean [16] and Asian Indian [17] populations. There are two possible mechanisms for this statistical association between the HLA haplotype and Takayasu arteritis as follows: (1) Disease-associated HLA molecules themselves determine susceptibility to the
disease by controlling immune responsiveness to self- or non-self-antigen(s) which trigger the disease. (2) Unknown gene(s) in strong linkage disequilibrium with the disease-associated HLA haplotype control susceptibility to the disease. At this stage, we can not distinguish between these two possibilities. To test the former hypothesis, it is essential to identify antigenic peptide(s) bound to disease-susceptible HLA molecules which activate T cells responsible for the development of the disease.

It was hypothesized that human T cells do not acquire tolerance to some disease-associated self-peptide(s), in the context of disease-susceptible HLA-II molecules in patients with autoimmune diseases. Some autoimmune diseases are caused by production of autoantibodies stimulated by autoreactive Th2 (helper T) cells and others are caused by tissue destructions induced by immune responses of autoreactive Th1 (inflammatory T) cells [18]. In this paper, to provide suggestive methods for further analysis of HLA-associated susceptibility to Takayasu arteritis, we summarize our recent molecular analyses on HLA-II-associated susceptibility to MS [19], IDDM [20] and infant-onset MG [21]. The manipulation in vitro of human CD4+ T cell response by analogues of the antigenic peptides is also described [21,22].

2. Existence of a clinically and immunogenetically unique subtype of multiple sclerosis (MS) in the Japanese population

MS is thought to be an autoimmune disease caused mainly by autoreactive Th1 (inflammatory T) cells specific to myelin-derived proteins which are produced by oligodendrocytes. This hypothesis comes from investigations of an experimental autoimmune encephalomyelitis in mice or rats and patients with MS in humans (see review in [23]). It was well noticed by many neurologists that there was a unique clinical subtypes (Asian-type) of MS in the Asian populations [24]. The patients with Asian-type MS show a relapsing–remitting course and have severe lesions in the spinal cord and optic nerves with a relatively small number of brain lesions [25]. We recently found that Asian-type MS is distinct from Western-type MS, not only in clinical manifestations but also in a HLA-linked genetic background.

It has been well documented that the susceptibility to MS is associated with a HLA-DRB1*1501 (DR2 subtype)-DQ6 haplotype in Caucasians [26,27]. Moreover, HLA-DRB1*1501 or DQ6-restricted autoreactive T cells specific to encephalitogenic myelin antigens, such as myelin basic protein (MBP) and proteolipid protein (PLP), were evidenced predominantly in MS patients carrying the DRB1*1501 allele [28,29]. In past studies of HLA in Japanese patients with MS [30–33], association between HLA-class II alleles and MS was controversial and was reported for DP4 [30,31], DR8 and DR52 [32], and DR2 and DQB1*0602 [33]. Moreover, such a strong association of DRB1*1501 with MS as observed in Caucasians has never been proven in the Asian populations, including the Japanese population, by HLA typing, at the DNA level. None of the previous studies on HLA of Japanese patients with MS have directed attention to the distinct subtypes of MS. Since clinical characterization of the Asian-type MS was done prior to the introduction of magnetic resonance imaging (MRI), we performed a combined HLA-DRB1, -DRB3 and -DRB5 allele typing and MRI study on the clinically distinct subtypes of MS in the Japanese population, in order to better define the MS subtypes in Asians [18] and we confirmed the results by analysing further patients.

According to the clinical findings, 44 patients who showed a relapsing–remitting course and a selective involvement of the optic nerve and the spinal cord with minimal brainstem signs were classified as Asian-type MS. The other 46 patients with involvement of multiple sites in the central nervous system were classified as Western-type MS. Asian-type MS showed a significantly lower number of brain lesions on MRI than did Western type, while Asian-type MS showed a significantly higher frequency of gadolinium-enhanced lesions on the spinal cord MRI than did Western type. As shown in Table 1, among the DRβ chain genes examined, only the frequency of the DR2-associated DRB1*1501-DRB5*0101 haplotype was found to be significantly higher in Western-type MS (N=46, 37.0%, corrected \( p<0.05 \)) than in either Asian-type MS (N=40, 7.5%) or healthy controls (N=113, 14.2%). Heterogeneity in the immunogenetic background and in the MRI
features between the two subtypes of MS suggests the presence of two etiologically distinct MS types in the Japanese population. On the contrary, the frequency of HLA-DPB1*0501 allele was significantly increased in Asian-type MS (N=46, 89.1%, corrected p<0.05), but not in Western-type MS (N=46, 71.7%) as compared with that in healthy controls (N=113, 63.0%) [34]. The frequency of HLA-DPA1*0202 allele was also significantly increased in Asian-type MS (N=46, 91.3%, corrected p<0.05), but not in Western-type MS (N=46, 82.6%) as compared with that in healthy controls (N=92, 65.2%). Increase in frequency of both DPA1*0202 and DPB1*0501-positive subjects was statistically significant in Asian-type MS (84.8%, corrected p<0.05) but not in Western-type MS (67.4%), as compared with healthy control subjects (54.3%).

It is interesting and important to ask whether the Asian-type MS is also associated with autoimmunity to some protein(s) specifically expressed in the myelin sheath as is the case in Western-type MS [28,29]. If this is the case, there may be an autoantigen(s) unique to Asian-type MS, because tissue distribution of the inflammatory lesions is apparently different between two subtypes of MS. We started to investigate the frequencies of autoreactive T cells specific to MBP, PLP and other myelin-derived proteins among Asian-type MS, Western-type MS and healthy controls. If T cells autoreactive to these proteins are evidenced in higher frequency in Asian-

### Table 1

Association between HLA-DRB1*1501-DRB5*0101 haplotype and Western-type MS but not Asian-type MS in the Japanese population

<table>
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<th>DRB1*</th>
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<th>Western-type (n=46)</th>
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<sup>a</sup>The corrected p value is statistically significant between two MS subtypes.
<sup>b</sup>The corrected p value is statistically significant (<0.05) compared to the controls.
<sup>c</sup>Only the uncorrected p value is statistically significant (<0.05) between each MS subtype and the controls.
type MS as compared with healthy controls, it will be interesting to investigate whether autoreactive T cells are preferentially restricted by disease-susceptible HLA-DPA1*0202 and DPB1*0501 or not.

3. Peptide binding affected by polymorphism of HLA-DQβ57 controlling susceptibility to insulin-dependent diabetes mellitus (IDDM)

IDDM is thought to be an autoimmune disease caused mainly by autoreactive Th1 (inflammatory T) cells and cytotoxic T cells which attack insulin-producing β-cells of pancreatic Langerhans’s islands by the analyses of NOD mice, a murine model of IDDM, and patients with IDDM (see review in [35]). In Caucasians, HLA-DQ alleles encoding for non-aspartic acid (Asp) at DQβ57 such as HLA-DQ8 (DQA1*0302-DQB1*0302; DQβ57Ala) were strongly associated with susceptibility to IDDM [11,36], whereas those encoding for DQ8 were not. HLA-DQ9 (DQA1*0302-DQB1*0303; DQβ57Asp) is a common DQ allele in the Japanese population but not in Caucasians and differs only at β57 from HLA-DQ8 (DQA1*0302-DQB1*0302). It is a possible hypothesis that IDDM-susceptible but not IDDM-non-susceptible DQ may present autoantigenic peptides specific to β-cells to autoreactive T cells.

To identify the differential peptide binding affected by DQβ57, we compared binding capacities of many analogous peptides to DQ8 and DQ9 [20]. DQ9-binding peptides were identified by affinity-based selection of a phage random peptide library [37], using the biotinylated DQ9 complex purified from cell lysates of an EBV-transformed B-lymphoblastoid cell line. Non-conservative single residue substitutions at the underlined residues of a peptide ([KLDPDYVLWSSSTVVGLGAAGA][21]), which bound equally to DQ8 and DQ9 molecules with high-affinity, significantly decreased the peptide binding to both DQ8 and DQ9 suggesting that these residues are important for binding to DQ molecules. Binding capacities of the wild-type 21-mer, its truncated and poly-alanine-based derivatives KDYVLWSSSTV[13] and KAYAADAAAX[13] to DQ8 were the same as those for DQ9. Thereafter, by utilizing the peptide KDYVLWSSSTV[13] and its analogues, we compared the structural requirements for peptides bound to DQ8 and DQ9 molecules.

As shown in Fig. 2, the KDYVLWSSSTV[13]-based analogue peptides with substitutions at Thr showed that amino acids R, K, H, E, D, Q, N, T, S, V, L, I, F, M, W and Y bound to DQ8, whereas only R, T, V, L, I, F, M, W and Y bound to DQ9. Thus, significant differences exist between DQ9 and DQ8, in that the majority of polar residues regardless of their static charges at residue 12 bound to IDDM-susceptible DQ8, but not to DQ9. The affinities of KDYVLWSSSTV[13] and KAYAADAAAX[13] (where X is T, A, K, D or I) were almost equal to DQ8 and DQ9, suggesting that DQ8- and DQ9-binding peptide motifs can accept both the 8-mer and 9-mer frames, depending on the P2–P7 sequences.

Fig. 2. Binding spectrum to DQ8 or DQ9 molecules of KDYVLWSSSTV-based single amino acid-substituted peptides. The C-terminal anchor 'T' of the (KYVLWSSSTV) peptide was replaced by various amino acids and their IC50 values (μM) against the binding of 125I-labeled peptide (‘KDYVLWSSSTVGLGAAGA’[21]) with DQ molecules were determined. Binding capacity of peptides is expressed as 1/IC50. Higher 1/IC50 values indicate stronger binding capacities to DQ molecules. Open squares with a solid line indicate binding to DQ8 and closed diamonds to DQ9. Amino acids which created a difference in DQ binding capacity between DQ8 and DQ9 are boxed in aspartic acid (Asp) at DQβ57 such as HLA-DQ8 As shown in Fig. 2, the KDYVLWSSSTV[13] -based analogue peptides with substitutions at Thr showed that amino acids R, K, H, E, D, Q, N, T, S, V, L, I, F, M, W and Y bound to DQ8, whereas only R, T, V, L, I, F, M, W and Y bound to DQ9. Thus, significant differences exist between DQ9 and DQ8, in that the majority of polar residues regardless of their static charges at residue 12 bound to IDDM-susceptible DQ8, but not to DQ9. The affinities of KDYVLWSSSTV[13] and KAYAADAAAX[13] (where X is T, A, K, D or I) were almost equal to DQ8 and DQ9, suggesting that DQ8- and DQ9-binding peptide motifs can accept both the 8-mer and 9-mer frames, depending on the P2–P7 sequences.

Residues at the C-terminal moiety of the peptide made for binding to DQ molecules, i.e., the 8th (P8) or the 9th (P9) residue from the most N-terminal anchor residue capable of binding, differed between DQ8 and DQ9 molecules. All the polar residues capable of participating in hydrogen bonding through side chains made for binding with DQ8 irrespective of their static charges, thereby indicating the involvement of hydrogen bonds between DQ and P8(P9) residue of the peptide. In general, it may be
that IDDM-susceptible DQ8 molecules can bind more species of peptides than IDDM-non-susceptible DQ9 molecules.

Several proteins expressed in β-cells of pancreatic Langerhans’s islands have been identified as disease-responsible autoantigens in the NOD mice, a model of IDDM, i.e., GAD65, heat-shock protein 60 (HSP60), carboxypeptidase H (CPH), peripherin, insulin and 37/40KD [38,39]. We, therefore, searched for DQ-binding peptide motifs in these proteins with possible binding to IDDM-susceptible DQ8 but not to DQ9. Assuming that only hydrophobic residues at the 1st and 2nd anchor positions are used for binding to DQ8 and DQ9 molecules, as observed in other DQ motifs [40-43], many peptide fragments were expected to bind to DQ8 but not to DQ9, (GAD65; 58 fragments, HSP60; 47 fragments, CPH; 42 fragments, peripherin; 34 fragments, insulin; 12 fragments, 37/40KD; 59 fragments). Among these, GAD65 is suggested to be the most likely autoantigen initiating β cell-specific autoimmunity in IDDM [38,39]. A limited fragment (p254–269: ARFKMFPEVKEGMAA) of GAD65 stimulated proliferative responses in patients’ T cells [44] and has homology with Cox P2-C peptide (p32–47: LKVKILPEVKHEFL) derived from Coxsackie viral protein [45], which suggests that molecular mimicry between these two peptides may be the mechanism governing the development of IDDM after infection with Coxsackie virus. Therefore, we investigated binding between DQ molecules and the GAD65p254–269 peptide (ARFKMFPEVKEGMAA) and Cox P2-Cp32–47 peptide (LKVKILPEVKHEFL) in which putative DQ-anchor residues were underlined. Both peptides weakly bound to DQ8 molecules and IC₅₀ values were 350 μM and 500 μM, respectively, where a small IC50 value indicates strong binding capacity of the peptide. On the contrary, both peptides hardly bound to DQ9 molecules and IC₅₀ values were 814 μM and 1036 μM, respectively. It may be that patients’ T cells do not acquire tolerance to GAD65 (p254–269) peptide presented by DQ8 because of low density expression of the HLA-DQ+peptide complexes on the surface of β cells or APC engulfed β cells. When the expression level of DQ8+GAD peptide complexes is increased for some unknown reasons, these GAD autoreactive T cells may be activated to directly or indirectly attack β cells. On the other hand, DQ9 molecules bind too small an amount of GAD peptide to activate autoreactive T cells on any occasion. To verify this hypothesis, it is essential to identify GAD epitope(s) and the antigen-presenting HLA-II molecules of GAD autoreactive T cells in many Caucasian patients with IDDM.

As for the IDDM-susceptible HLA-II alleles in the Japanese population, HLA-DRB1*0405-DQ4 is strongly and HLA-DRB1*0901-DQ9 is weakly associated with susceptibility to IDDM [46]. It is important to note that the frequency of HLA-DQ alleles having DQβ₅⁷ non-Asp is very low in the Japanese population, and that DQβ₅⁷ of both IDDM-susceptible DQ4 and DQ9 is Asp [47] which is a characteristic of IDDM-non-susceptible HLA-DQ in Caucasians. This may be one possible reason for the relatively low incidence of IDDM in the Japanese population. As DQβ₅⁷ non-Asp-associated susceptibility to IDDM is not applicable to Japanese IDDM, one will need to identify autoimmune self-peptide(s) and their antigen-presenting HLA-II molecules in the Japanese patients to elucidate the mechanism for HLA class II-associated susceptibility to Japanese IDDM. Our current investigation revealed that GAD65-autoreactive T cells exist in Japanese patients with IDDM and that they were not restricted by HLA-DQ but are restricted by-products of the HLA-DR gene family or HLA-DP molecules [48].

4. Modulation of human T cell responses by analogues of antigenic peptides

The CD4⁺ T cells recognize non-self-peptides in the context of self-HLA class II molecules and it was considered that recognition and response of T cells were apparently an on/off phenomenon. However, findings in mice utilizing analogue peptides carrying single residue substitutions in antigenic peptides revealed that T cell clones recognize these altered peptide ligands to show altered T cell response. Altered peptide ligands induced T cell non-responsiveness through TCR antagonism [49,50] or induction of anergy as a consequence of partial activation [51,52], and sometimes induced dissociation between proliferative response and cytokine production [53,54]. Some TCR antagonistic analogue peptides 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 [51]. These phenomena in human T cells were recently well characterized by us and others [21,22,55–61].

Once self-peptides which trigger disease-related autoreactive T cells are identified in patients with autoimmune diseases, it will be of interest to down-regulate responses of pathogenic autoreactive T cells by analogues derived from autoantigenic peptides and having TCR antagonistic or TCR partial agonistic activities [55]. Another important study is to identify non-self-peptides which stimulate autoreactive T cells by molecular mimicry to autoantigenic peptides, as suggested in cases of autoimmunity to myelin basic protein in patients with MS [29]. If we know the general rule for the structures of analogue peptides derived from antigenic peptides, which stimulate or inhibit T cell responses to those wild-type antigenic peptides, it will be easier for us to identify structures of peptides which cross-activate or inhibit responses of autoreactive T cells. For this aim, we analyzed responses of a human T cell clone to a large panel of analogue peptides derived from a non-self-antigenic peptide presented by Asians-specific DR4 (DRB1*0406) which is associated with susceptibility to insulin autoimmune syndrome (Hirata’s disease), a disease unique to Asian populations [14].

A CD4+ human T cell clone YN5-32 recognized a streptococcal M12p54-68 peptide in the context of HLA-DR4 (DRA+ DRB1*0406) and produced a large amount of IFNγ and a small amount of IL-4. We investigated responses of YN5-32 to 156 independent analogue peptides carrying single residue substitutions at residues 57(P1)–65(P9) of the peptide where P1 (position 1) means the putative most N-terminal DR anchor residue [22]. As shown in Fig. 3, the residues Leu-57(P1), Ala-60(P4) and Asn-62(P6) were the most likely to be DR-anchor residues, and 30% (17/57) of analogue peptides 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 non-full agonistic peptides exhibited TCR antagonism. On the other hand, the 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, indicating that substitutions at these residues frequently abrogate T cell recognition. Interestingly, as many as 60.4% (29/48) of non-full agonistic analogues exhibited TCR antagonism to inhibit proliferative response of YN5-32 to the wild-type peptide. TCR antagonistic activity of the analogue peptides proves binding of those peptides to HLA-DR molecules. Therefore, up to 76.2% of analogues substituted at one of these three residues binds to HLA-DR indicating that three residues must not be DR-anchor residues. Thus, analogues of the antigenic peptides substituted at TCR-recognition residues are good candidates for immuno-suppressive peptides. We applied these findings to a search for inhibitory analogue peptides specifically acting on autoreactive T cells responding to acetylcholine receptor (AChR) and established from a patient with myasthenia gravis (MG) as described below.

5. Immuno-suppressive peptides acting on a T cell clone autoreactive to acetylcholine receptor (AChR)

MG, an autoimmune disease accompanied by IgG-
class autoantibodies to the AChR expressed at the neuromuscular junction. Anti-AChR autoantibodies in MG patients cause accelerated degradation of the AChR triggered by AChR crosslinking or by complement-mediated lysis of the postsynaptic membrane, and block the cholinergic site [62,63]. Because CD4+ helper T cells are essential for induction of IgG production by B cells, CD4+ T cells autoreactive to AChR have a crucial role for development of MG in an experimental autoimmune MG in mice [64].

Distribution of the age at onset of Japanese MG patients shows bimodal peaks: the first and the highest peak is seen before 3 years of age and the second lower peak occurs in the third decade of life [65]. In contrast, in Caucasians, the age at onset of MG generally shows a single peak in adulthood [66], in which susceptibility to MG is associated with HLA-DR3 and HLA-B8 [67,68]. In the Japanese patients with MG who developed MG before 3 years of age, the frequencies of HLA class II haplotypes common in the Asian populations, HLA-DR9(DRB1*0901)-DQ9(DQA1*0301-DQB1*0303), DR13(DRB1*1302)-DQ6(DQA1*0102-DQB1*0604) and their double-heterozygotes were significantly increased [69,70]. The clinical manifestations of myasthenia are also different between infant-onset MG and adult-onset MG. Infant-onset MG has a relatively benign prognosis and affects mainly ocular muscles without causing severe and generalized muscle weakness.

The AChR of mature muscle consists of five subunits in the stoichiometry two α, β, δ and ε subunits [71]. The AChRα subunit exists in two isoforms, with or without a peptide encoded for by exon P3A which can be alternatively spliced [72,73] and their functional difference is unknown. The binding site of the anti-AChR auto-antibodies locates at an extracellular region of the α subunit, designated as the main immunogenic region [74]. The acetylcholine binding sites consist of α-γ(ε) and α-δ complexes, and epitopes recognized by CD4+αβ autoreactive T cells in AChR α, β, γ and δ subunits have been noted in MG [75–78].

To elucidate mechanisms involved in susceptibility to infant-onset MG associated with HLA class II alleles, we established and analyzed an AChRα-autoreactive CD4+αβ T cell clone from a Japanese patient with infant-onset MG. We then analyzed the effect of analogue peptides derived from an autoantigenic peptide, on the immune responses of the autoreactive T cell clone [21]. An AChRα peptide (p71–91) specific autoreactive CD4+αβ T cell clone was established by stimulating peripheral blood mononuclear cells from a patient heterozygous for two disease-susceptible HLA-DR9-DQ9 and DR13-DQ6 haplotypes with a mixture of overlapping peptides covering acetylcholine receptor α-subunit (AChRα). The T cell clone recognized the AChRα peptide in the context of the HLA-DQ6 molecule and produced a large amount of IFN-γ and a trace amount of IL-4 showing Th1-like phenotype. The majority (p75–83) of the core epitope of the autoantigenic peptide (p75–87) is encoded for by an exon P3A of the AChRα gene which can be alternatively spliced (Fig. 4). In Caucasian MG, T cells autoreactive to this AChRα epitope were scarcely reported [79]. The T cell clone responded to the recombinant AChRα protein with a P3A exon product, but not without a P3A exon product.

![Fig. 4. Characteristics of a T cell clone autoreactive to AchRα peptide in the context of disease-susceptible HLA-DQ6 molecule and established from a Japanese patient with infant-onset myasthenia gravis. The autoreactive T cell clone recognized an AchRα p75–87 autoantigenic peptide and the N-terminal side of the peptide indicated by hatched bars, p75–83, is encoded for by the P3A exon which can be alternatively spliced. The T cell clone had a Th1-like phenotype to produce a large amount of IFN-γ and a trace amount of IL-4.](image-url)
We investigated responses of the T cell clone to 114 analogue peptides carrying single residue substitutions in the core AChRα peptide. The majority of analogues substituted at residues Phe-77, Leu-80 and Asn-82 stimulated proliferation of the T cell clone. Conversely, the majority of analogue peptides substituted at either Gln-81 or Glu-83 did not stimulate proliferative responses suggesting that these two residues may be TCR contact sites, and all analogues exhibited strong or intermediate inhibitory effects on proliferative responses of the T cell clone to the wild-type peptide, probably by TCR antagonism (Fig. 5). Thus, an HLA class II allele common in Asians may directly control susceptibility to Asians-specific clinical subtype of myasthenia gravis. Analogues of auto-antigenic AChRα peptide may prove effective for new immuno-suppressive therapy.

6. Conclusions

In summary, we described our strategy for elucidation of mechanisms for HLA-class II-associated susceptibility to autoimmune diseases including MS, IDDM and infant-onset MG. In the Japanese population, the susceptibility to all of these diseases is strongly associated with particular HLA-DR-DQ haplotypes unique to or common in the Asian populations, and clinical features of some of these diseases are different between Caucasians and Asians. We identified differences in binding-peptide motifs between IDDM-susceptible HLA-DQ8 and non-susceptible HLA-DQ9 molecules. A unique autoantigenic epitope presented by disease-susceptible HLA-DQ6 to autoreactive T cells was identified in a patient with infant-onset MG. Our strategy is useful to identify autoimmune self-peptides, and it is suggested that not only disease-susceptible HLA class II but also self-peptides causing diseases are different between Caucasians and Asians. These differences may well correlate to different clinical manifestations of diseases between the two ethnic groups. Analyses of responses of human T cell clones specific to either an autoimmune self-peptide or a non-self-peptide to a large panel of analogue peptides revealed the frequent appearance of immuno-suppressive analogues which inhibit T cell responses to the wild-type peptides. This kind of altered peptide ligands may be useful for down-regulation of responses of human pathogenic autoreactive T cells.

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