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Degenerate recognition and response of human CD4$^+$ Th cell clones: implications for basic and applied immunology

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Abstract

It was once considered that the T cell response is an all or nothing type event, but recent studies have clearly indicated that T cells show many different types of activation in recognition of altered ligands for T cell receptors (TCR). In this review, we summarize our recent findings on the response of human CD4$^+$ helper T (Th) cell clones to altered peptide ligands (APL); peptides carrying single or multiple residue substitutions in antigenic peptides. The extensive analyses revealed that TCR-antagonism and partial agonism are frequently observed by the stimulation with APLs substituted at particular amino acid residues of antigenic peptides. We observed unique partially agonistic APLs inducing prolongation of T cell survival without cell proliferation. Superagonistic APLs stimulated enhanced proliferation and production of cytokines in Th cell clones reactive to tumor-associated antigens. The other APL induced enhanced production of interleukin-12 by antigen presenting cells and subsequent enhancement of IFN-γ production by T cells reactive to allergens. By utilizing an HLA-DR-restricted T cell epitope library generated by mutated invariant chain genes, it was revealed that human Th cell clones recognize a more diverse array of peptides with multiple and simultaneous amino acid substitutions in an antigenic peptide. APLs also induced altered intracellular signaling events including intracellular calcium increase and phosphorylation of signaling molecules. This information provides basic knowledge regarding the characteristics of antigen recognition by human Th cells and the subsequent activation, and a novel method for manipulation of human Th cell responses by APLs, as a possible candidate for antigen-specific immuno-potentiating or immunosuppressive therapy.

Keywords: HLA class II molecule; Human CD4$^+$ Th-cells; Antigenic peptides; Altered peptide ligands; T cell recognition; TCR antagonism; Peptide partial agonism

1. Introduction

The human histocompatibility leukocyte antigen class-II (HLA-II) molecule has a peptide binding groove on top of the molecule and binds antigenic peptides processed by antigen presenting cell (APC) to present them to CD4$^+$ helper T (Th) cells (Germain and Margulies, 1993). Three to five amino acid residues were separated by one to two intervening residue(s) and acted as anchor residue(s) for peptide binding to HLA-II molecules (Sette et al., 1993; Hammer et al., 1993; Matsushita et al., 1994). On the other hand, side chains of amino acid residues flanking anchor residues proved to be the main recognition sites by T cell receptors (TCR); this was clearly established in crystallographic analyses of the DR molecule bound by either self (Brown et al., 1993) or non-self peptides (Stern et al., 1994).

CD4$^+$ Th cells usually recognize non-self peptides in the context of self HLA-DR molecules. Recognition and responses of T cells were once considered to be an on/off phenomenon, however recent findings obtained using altered peptide ligands (APLs) carrying single residue substitutions in antigenic peptides presented by one major histocompatibility complex (MHC) class II molecule or one specific peptide presented by different MHC class II molecules showing a limited polymorphism revealed that

Abbreviations: HLA-II, human histocompatibility class II; APC, antigen presenting cell; Th cell, helper T cell; TCR, T cell receptor; APL, altered peptide ligand; MHC, major histocompatibility complex; IFN-γ, gamma interferon; IL, interleukin; CLIP, class II-associated invariant chain peptide; ZAP-70, zeta-associated protein-70

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altered TCR ligands induce altered T cell responses in both mice and humans, including (1) T cell non-responsiveness, through TCR antagonism and (2) partial agonism inducing partial activation of T cells without cell proliferation (Sloan-Lancaster and Allen, 1996).

Previous analyses also revealed that the interactions of TCRs with MHC-wild-type peptides had stronger affinities and/or smaller off-rates than did those of TCRs with MHC-APL complexes (Lyons et al., 1996). These differences in characteristics of molecular interactions may induce insufficient engagements of TCR with MHC-APL complexes such that intracellular signals mediated by TCR through recognition of APLs are inadequate for full activation of T cells to induce cell proliferation. In some cases, inadequate signals induce unique altered T cell responses.

In this review, we will summarize our recent analyses on recognition by human $CD4^+$ Th cell clones of diverse peptides, and the heterogeneity of subsequent T cell responses and T cell activation signals induced, as summarized in Table 1.

2. Frequencies of agonistic and antagonistic single residue substituted APLs depend on position of substituted amino acid residues of the peptide

If there is a general rule for structures of APLs which stimulate or inhibit T cell responses to wild type antigenic peptides, it would be easier to generate peptides which augment or inhibit responses of human Th cells. We used a human Th1-cell like clone YN5-32 reactive to a streptococcal M12p54-68 peptide (^{54}NRDLEQAYNELSGEA^{68}) in the context of HLA-DR4 (DRB1*0406), and analyzed responses of YN5-32 to 156 independent APLs 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 (Chen et al., 1996). As shown in Fig. 1, 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 APLs substituted at these residues exhibited full agonism to stimulate various magnitudes of proliferative responses in the T cell clone, whereas only 7.5% (3/40) of non-fully agonistic peptides exhibited TCR antagonism. On the other hand,

Table 1
Summary of our observations on responses of human $CD4^+$ Th-cell clones to APLs

Th-cell clone	Specificity	Observed immune responses to APLs	Reference
YN5-32	Streptococcal M12p54-68/DR4	TCR antagonism	
		Partial agonism; increases in cell size and expression levels of CD4, 11a, 28, 49d, 95 without anergy induction	Chen et al. (1996)
		Polymorphism at DRβ37 affected T cell recognition	Chen et al. (1997)
		Quantitative and qualitative alteration of intracellular calcium increase	Chen et al. (1998)
		Overexpression of partially agonistic TCR-ligand induced proliferation without phosphorylation of ZAP-70 and LAT	Irie et al. (2003)
SK2.11	AChRα p75-87/DQ6	TCR antagonism	Kanai et al. (1997).
BC20.7, BC33.5, BC42.1	BCGa p84-100/DR14	Partial agonism in recognition of artificial or natural self APLs; increased survival without antigenic stimuli or production of lL-4 and lFN-γ without cell proliferation	Matsushita et al. (1997)
C27	p21Ras p3-17/DR1	Superagonism; increased proliferation and production of IFN-γ and GM-CSF in recognition of cancer-associated mutated peptides and its APL	Yokomizo et al. (1997)
Y41.2	TEL/AML1 fusion peptide/DP17	Superagonism; increased proliferation and production of IFN-γ and GM-CSF in recodnition of APLs derived from leukemia-associated TEL/AML1 fusion peptide	Yun et al. (1999)
29.15.2	p21Ras p3-20/DR51	Superagonism; increased proliferation in recognition of APL identified by using a combinatorial peptide library and mass spectrometry	Tanaka et al. (1999)
ST1.9	*Cry j*Ip335-346/DR52	Superagonism; increased production of IFN-γ	Ikagawa et al. (1996)
DT13.2	*Der f*Ip18-31/DQ6	Superagonism; increased production of IFN-γ stimulated by increased production of IL-12 from antigen presenting cells	Matsuoka et al. (1996)
SA32.5, MK20.2	GAD65 p115-127/DR53	Generation of a multiple residue substituted epitope expression library by using CLIP-substituted invariant chain genes to identify agonistic APLs and mimicry microbial peptides	Uemura et al. (2003)

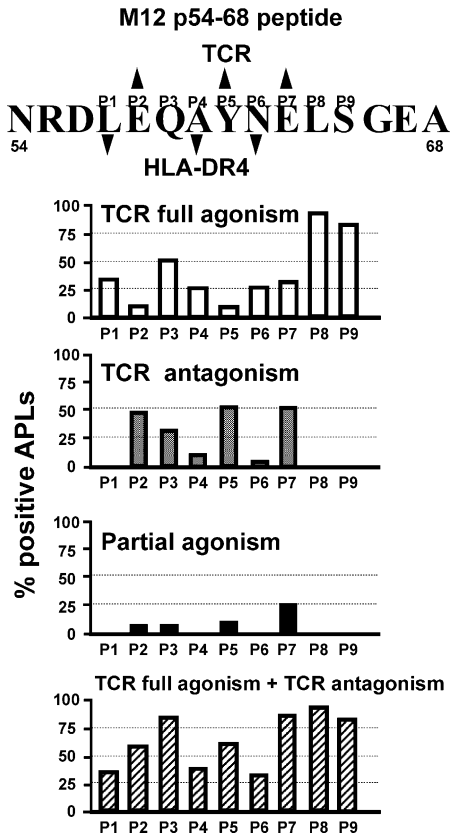

Fig. 1. Summary of responses of the human Th cell clone YN5-32 to 156 APLs carrying single residue substitutions in a streptococcal antigenic peptide M12p54-68. From P1 to P7 residues, residues were replaced with 19 other amino acids. The P8 and P9 residues were replaced with 10 and 11 other amino acids, respectively. Percentages of APLs exhibiting either full agonism (open bars), TCR antagonism (shedded bars) or partial agonism (closed bars) are indicated for each residue. APLs carrying substitutions at putative TCR contact residues, P2, P5 and P7, frequently exhibited TCR antagonism. Some of them, especially APLs substituted at P7, exhibited partial agonism. Because APLs with full agonism or TCR antagonism have to bind to MHC molecules, the frequencies of those peptides indicated by cross hatched bars represent the frequency of peptides with MHC-binding capacity.

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 APLs stimulated proliferative responses in YN5-32 thereby indicating that substitutions at these residues frequently abrogate T cell recognition. Interestingly, as many as 60.4% (29/48) of non-fully agonistic APLs exhibited TCR antagonism to inhibit the proliferative response of YN5-32 to the wild-type peptide.

Eight (27.6%) of these antagonistic APLs carrying relatively conservative amino acid substitutions exhibited partial agonism to induce large increases in cell size and expression levels of CD4, CD11a (LFA-1α), CD28, CD49d (VLA-4α) and CD95 (Fas), on the T cell surface, as compared with responses to the wild-type peptide. This was the most prominent at Glu-63 (p7) where 5 of 10 antagonistic APLs exhibited partial agonism. These observations indicate that many APLs carrying substitutions at TCR recognition sites in the T cell epitope induce a partial agonism as well as TCR antagonism in YN5-32, as noted by other studies of mouse T cell clones. Differences, such as the absence of anergy induction or little increase in CD25 expression by partially agonistic APLs have been noted in human Th cells. The polymorphism (Ser-Tyr) at the DRβ^{37} residue induced conformational changes of peptides, which can be distinguished by YN5-32 TCR in some but not all peptides, providing further evidence for altered human T cell responses induced by minor modifications of TCR ligands (Chen et al., 1997).

Based on this knowledge of Th cell responses to APLs, we identified many antagonistic APLs which can inhibit proliferation of Th-cell clones auto-reactive to the acetylcholine receptor α chain derived self peptide in the context of the disease-susceptible HLA-DQ6 molecule and established from a patient with infant-onset myasthenia gravis unique to Asian populations (Kanai et al., 1997).

3. Unique partially agonistic APLs inducing prolonged survival of Th cells in the absence of antigenic stimulus

By utilizing three other human Th cell clones with distinct TCR-Vβ recognizing the same non-self mycobacterial *Bacillus Calmette-Guérin* a (BCGa) peptide/HLA-DR14 complex, we found another type of unique partial agonism, as follows (Matsushita et al., 1997). Stimulation of T cells with a one-residue-substituted APL or a minimally homologous self-peptide fragment can prolong the in vitro survival of T cells in the absence of antigenic stimuli, in a clone specific-manner. This prolongation is associated with the up-regulation of Bcl-x_L, without proliferation and these peptide-clone combinations are capable of inducing lymphokine secretion. Thus, peptide partial agonism may play a role in the survival of not only thymocytes but also mature Th cells, in the absence of non-self peptide ligands.

4. Augmentation of T cell responses (superagonism) stimulated by APLs: implication to peptide-based cancer immuno-therapy

A T cell response to a tumor requires a tumor antigen processed into peptides which can be presented to $CD8^+$ cytotoxic T cells by MHC class I molecules, and to $CD4^+$ Th cells by MHC class II molecules. While cytotoxic T cells can kill tumor cells directly, some Th1 cells can mediate cytotoxicity to tumors, amplify responses of cytotoxic T cells, and activate APC, through secretion of lymphokines to augment anti-cancer immunity. We established a Th cell clone reactive to oncogenic and mutated p21 Ras proteins as well as mutated peptides, in an HLA-DR1-restricted manner. We provided evidence for augmentation of proliferation and production of gamma interferon (IFN-γ) and granulocyte-macrophage colony-stimulating factor (GM-

CSF) by this T cell clone in recognition of APLs carrying a single residue substitution in the mutated P21 Ras peptide (Yokomizo et al., 1997).

We also identified superagonistic and single residue substituted APLs derived from leukemia-associated TEL/AML1 fusion peptide (IGRIA/ECILGMNPSR) (Yun et al., 1999). The APLs having Val or Leu substitutions at putative P8(Gly) or P9(Met) of the peptide respectively stimulated much stronger proliferation and production of Th1-type cytokines in a Th clone reactive to TEL/AML1 fusion peptide in the context of HLA-DP17. These superagonistic APLs can be given consideration for anti-leukemic immunotherapy.

To identify peptide superagonists in a systematic and sophisticated manner, we used a combinatorial peptide library and mass-spectrometry (Tanaka et al., 1999). The proliferative responses of a human $CD4^+$ T cell clone reactive to a self-K-Ras-derived peptide, Ras p3-20 (^{3}EYKLVVVGAGGVGKSALT^{20}), were tested using a set of X9 combinatorial peptide libraries containing the flanking residues (EYKLVXXXXXXXXXSALT, where X indicates random amino acids). Certain peptide libraries, such as EYKLVXXXXXXXMXXSALT and EYKLVXXXXXXXHXSALT, stimulated a marked proliferation of T cells. However, no combinations of substitutions tested, such as EYKLVXXXXXXMHXSALT, exhibited additive effects. We subsequently synthesized peptides with degenerate sequences (a mixture of 480 species), where each position is composed of the wild-type residue or of amino acids that induced the proliferation of T cells, in positional scanning. Interestingly, one fraction of degenerate peptides, separated by reverse-phase HPLC, stimulated a much stronger proliferation than did the Ras p3-20; in addition, the retention time of this fraction was distinct from that of Ras p3-20. Mass spectrometry analysis of this fraction and flanking fractions identified five peptide species that exhibit strong signals in a manner that parallels the antigenic activity. Finally, 17 candidate peptide sequences were deduced from mass spectrometry and hydrophobicity scoring results, of which two peptides (EYKLVVVGAGGMLKSALT and EYKLVVVGAGGMIKSALT) did induce 52- and 61-fold stronger proliferation, respectively, compared with the Ras p3-20. These findings indicate that: (1) synthetic peptides that carry "the best" residue substitution at each position of combinatorial peptide libraries do not always exhibit superagonism, and (2) such a drawback can be overcome with the use of mass spectrometry. This approach provides new perspectives for accurate and efficient identification of peptide superagonists.

5. APL affects not only T cell responses but also APC responses to increase IL-12 production: Implication to peptide therapy inducing Th1-dominance

Human Th0 clone DT13.2 reactive to the group I allergen in *Dermatophagoides farinae* extracts (*Der f* I) p18-31 (^{18}RSLRTVTPIRMQGG^{31}) in the context of HLA-DQ6 (DQA1*0102/DQB1*0602) molecules was generated from a patient with bronchial asthma and DT13.2 produced both interleukin (IL)-4 and IFN-γ. Analysis of changes in DT13.2 responses to *Der f* I p18-31-derived APLs revealed that the substitution of ^{27}Arg to Lys resulted in a significant increase in IFN-γ production, with no remarkable changes either in proliferative response or in IL-4 production (Matsuoka et al., 1996). Interestingly, the selective enhancement of IFN-γ by the APL was accompanied by an increased production of IL-12 and this event was suppressed by an anti-IL-12 antibody down to the level of IFN-γ production induced by the wild-type peptide. The superagonistic APL derived from another Japanese Cedar pollen allergen (*Cry J*I) also augmented production of IFN-γ in a human Th0 clone reactive to *Cry J*I peptide/HLA-DR52 complex (Ikagawa et al., 1996).

Our observations suggest that the mode of interaction between TCR and MHC/peptide complex may determine the Th1-predisposing condition by controlling the IL-12 production by APC. Furthermore, this kind of Th1-response inducing APLs may provide peptide therapy for diseases caused by Th2 responses such as allergy.

6. Generation of a Th cell epitope expression library for extensive analysis of degeneracy in peptides recognized by human Th cell clones

Because we found that the systematic detection of cross-recognized epitopes considering the combinatorial effect of amino acids within the epitope is impossible in approaches using positional scanning synthetic combinatorial peptide libraries, we established an alternative method by utilizing molecular genetic approaches. A DNA-based randomized epitope library using class II-associated invariant chain peptide (CLIP)-substituted invariant chains was generated (Fujii et al., 1998; Fujii et al., 2001; Uemura et al., 2003). This approach, by which multiple residues of an antigenic peptide were simultaneously randomized, has the great advantage of producing several conformations of the peptide/HLA-II complexes, and increasing the possibility to identify degenerate sequences with agonistic properties. GAD65-autoreactive T cell clones restricted by disease-susceptible HLA-DR53 and established from patients with type I diabetes were utilized as models. Analysis of agonistic epitopes indicate that recognition by each TCR was significantly affected by combinations of amino acids in the antigenic peptide, although the degree of combinatorial effect differed between each TCR. Protein database searching based on the TCR recognition profile proved successful in identifying several microbial and self-protein-derived mimicry epitopes with limited sequence homology to the original GAD65 epitope. Some of the identified mimicry epitopes were actually produced from recombinant microbial proteins by APCs to stimulate T cell clones. Our data

demonstrate the importance of the combinatorial nature of amino acid residues of epitopes to investigate diversity of T cell recognition and molecular mimicry, and the Th cell epitope display library we established provides a useful tool for these objectives.

7. Altered intracellular signalings induced in a Th-cell clone by APLs

In mouse T cell clones, TCR antagonistic or partially agonistic APLs induce partial phosphorylation of CD3ζ chains leading to the absence of phosphorylation and activation of ZAP-70 (Sloan-Lancaster et al., 1994; Madrenas et al., 1995). Studies of calcium signaling activity in mouse T cells stimulated with APLs indicated that the Ca^{2+} response induced by antagonistic APLs was smaller in amplitude and shorter in duration than that induced by fully agonistic ligands (Sloan-Lancaster et al., 1996; Wülfing et al., 1997).

To determine if APLs affect intracellular activation signals in human Th cells, we investigated changes in intracellular calcium concentrations ($[Ca^{2+}]_i$) in the Th cell clone YN5-32 stimulated with either fully agonistic peptide M12p54-68 or partially agonistic APL E63V (standing for APL having Val-substitution at amino acid residue 63 Glu), or simply antagonistic APL E58M as described in the Section 1 (Chen et al., 1998). Both E63V and E58M stimulated a Ca^{2+} response in ∼40% of the T cells, whereas M12p54-68 did so in ∼70% of T cells. The most predominant pattern of a Ca^{2+} increase induced by M12p54-68 was a small sinusoidal peak followed by a sustained high response. The most frequent pattern of calcium response induced by E63V was a continuous high response without a preceding sinusoidal peak, whereas that induced by E58M was large with frequent oscillations. Furthermore, our results suggest that the Ca^{2+} response induced by the fully agonistic peptide depends on activation of the genistein-sensitive signaling pathway, including protein tyrosine kinases, whereas the Ca^{2+} response to a simple antagonistic APL completely depends on activation of the GF109203X-sensitive signaling pathway, including protein kinase Cs and extracellular Ca^{2+}. These differences in the $[Ca^{2+}]_i$ response in recognition of different APLs may parallel the unique T cell activation patterns induced by APLs in human T cells.

We then asked whether forced overexpression of partially agonistic TCR-ligands on APCs provides high-avidity TCR-ligands to stimulate T cell proliferation, we generated L cell transfectants expressing various numbers of HLA-DR4 covalently linked with APLs derived from M12p54-68 peptide and observed responses of the cognate T cell clone YN5-32. Some overexpressed HLA-DR4/partially agonistic APL complexes induced T-cell proliferation in a density-dependent manner, however tyrosine-phosphorylation of ZAP-70 and linker for activated T cells (LAT) and kinase activity of ZAP-70 were not detectable (Irie et al., 2003). Our data suggest the presence of an unique signaling pathway coupling TCR-ligation with T cell proliferation in a ZAP-70 less dependent manner, and this activation pathway is observed when TCRs are engaged with relatively low affinity TCR ligands expressed in high density on the surface of APC. This suggests that T cell activation signals are not uniform and they can be alternatively activated depending on binding characteristics between TCRs and their ligands.

8. Conclusions

In conclusion, we observed various kinds of responses to APLs in human Th cell clones, as summarized in Table 1, and the implications of our findings are as follows. (1) It is so far difficult to predict degeneracy of Th cell recognition in a given TCR by analyzing the past literature, and our Th cell epitope expression library using CLIP-substituted invariant chain genes will provide a breakthrough in this field. (2) Our findings may support the following ideas, (1) maintenance of Th cell survival (memory ?) by self APLs in the absence of stimuli with non-self peptides, (2) triggering of autoreactive Th cells by non-self agonistic APLs (molecular mimicry), and (3) a possible application of APLs to augmentation of desirable anti-microbial or anti-tumor immunity, or to inhibition of pathological immune responses such as allergy and autoimmunity. Our analyses of human Th cell responses to APLs have provided pertinent information on the basic immunology of human Th cell biology and also on the strategy for new methods for manipulation of antigen-specific responses of human Th cells.

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