Differential Effects of CD28 Engagement and IL-12 on T Cell Activation by Altered Peptide Ligands

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Differential Effects of CD28 Engagement and IL-12 on T Cell Activation by Altered Peptide Ligands

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To further our understanding of the mechanisms underlying the diverse effects of altered peptide ligands (APL) on T cell activation, we used a population of nonactivated spleen cells from mice that expressed a transgenic TCR specific for myelin basic protein Ac1-11 and peptide analogues that display either enhanced or decreased affinities for TCR/MHC to address the question whether APL-induced signaling through the TCR can regulate the capability of APC to activate T cells. We demonstrate that weak agonist APL are poor inducers of all aspects of the activation of both the responder T cells and the APC. Enhancement of the antigenic signal by augmenting the binding of the weak agonists to MHC reversed their defective activating capacity. Enhancement of costimulation by CD28 only resulted in augmentation of the capacity of the weak agonist APL to induce proliferation and IL-2/IL-3 production, but not CD40L or IL-12Rβ chain expression on T cells, CD80/CD86 expression on APC, IL-12 secretion, or IFN-γ production. Exogenous IL-12 promoted IFN-γ production in the presence of the weak agonists. These studies demonstrate that there is a critical threshold of antigenic signal required for full activation of the T cell-APC interactions needed for the differentiation of Th1 cells. The provision of excess costimulation can overcome some of the defects in T cell activation by weak agonists, but is insufficient to induce a sufficient level of CD40L expression needed for engagement of CD40 on APC with subsequent IL-12 production and induction of IL-12Rβ chain expression. The Journal of Immunology, 1998, 161: 6614–6621.
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Materials and Methods

Mice

Mice that express transgenic TCR Vβ8.2 and Vα4 chains specific for MBP Ac-11 in association with I-Aκ have been described previously (26–28) and were generously provided by Dr. Charles A. Janeway (Yale University School of Medicine, New Haven, CT). The mice used in these studies were housed under pathogen-free conditions in the National Institutes of Health animal facility. All of the mice used were 6–8 wk of age. The H-2b haplotype was introduced into the transgenic background by crossing with B10.PL mice (The Jackson Laboratory, Bar Harbor, ME).

Peptides

MBP Ac-11 and variants were synthesized and purified by HPLC by the Peptide Synthesis Laboratory at the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD. The amino acid sequences of the peptide used in this study have been described elsewhere (29, 30).

FACS analysis

Single cell suspensions of spleen and lymph nodes from the transgenic mice were stimulated with varying concentrations of peptide in vitro for 18 h; the cells were then harvested, washed, and incubated with the combination of phycoerythrin (PE)-labeled rat anti-mouse CD40L and FITC-labeled rat anti-mouse CD4; or FITC-labeled rat anti-mouse CD80 and CD86, and PE-labeled rat anti-mouse B220. The cells were analyzed by FACS (Becton Dickinson, San Jose, CA). The expression of CD40L on the CD4+ T cells was analyzed on the gated CD4+ population and the expression of CD80 and CD86 was analyzed on the gated B220+ population. All of the mAbs were purchased from PharMingen (San Diego, CA) except the PE-labeled rat anti-mouse CD4 (Becton Dickinson).

Cell culture and proliferation assay

Unseparated populations of spleen and lymph node cells (2 × 107/ml) from the transgenic mice were cultured with varying concentrations of native or variant peptide for varying times at 37°C in 96-well microtiter plates (Costar, Cambridge, MA) in RPMI 1640 supplemented with 10% FCS, 2 mM glutamine, 5 × 10−5 M 2-ME, and antibiotics. In some studies, CD4+ T cells (5 × 106/ml), purified using CD4 T cell subset columns (R & D Systems, Minneapolis, MN), were cultured with irradiated T cell-depleted spleen cells (1 × 106/ml) as APC. For measuring proliferation, [3H]Tdr was added at 40 h and the cultures were carried out for an additional 14 h; the plates were then harvested and analyzed by scintillation counting. Purified monoclonal hamster anti-mouse CD28 (10 μg/ml, Pharmingen), monoclonal rat anti-mouse IL-12 (10 μg/ml, C17.8; a gift of Dr. G. Trinity, Wistar Institute, Philadelphia, PA) purified by passing ascites over the protein G column, mouse rIL-12 (10 ng/ml R & D Systems), or control rat IgG (Jackson ImmunoResearch, West Grove, PA) were added at the beginning of the cultures. Supernatant from the cultures was collected at 24 h (IL-2) or 48–72 h (IFN-γ and IL-3) for measuring cytokines.

Cytokine ELISA

Cytokines were quantified using a standard ELISA technique based on noncompeting pairs of Abs. Purified rat anti-mouse IL-2, IL-3, and IFN-γ and biotin-labeled anti-IL-2, IL-10, and IL-3 were obtained from PharMingen. Rabbit anti-mouse IFN-γ was obtained from Spring Valley Laboratories (Woodbine, MD). Goat anti-rabbit IgG-horseradish peroxidase was obtained from Southern Biotechnology Associates (Birmingham, AL), and was used as a tertiary Ab in the IFN-γ ELISA. Peroxidase-labeled streptavidin (Kirkegaard & Perry Laboratories, Gaithersburg, MD) was applied as a last step in the ELISAs using biotin-labeled detection Abs. Plates were developed with TMB peroxidase substrate (Kirkegaard & Perry) and the reaction was stopped with 10% sulfuric acid.

Northern blot analysis

Total RNA was isolated from cultures of stimulated spleen and lymph node cells from the transgenic mice using RNAzol RNA isolation solvent (TEL-TEST, Friendswood, TX). Total RNA (5 μg) was used in each lane in 1% agarose gel and transferred to nitrocellulose membrane (Schleicher & Schuell, Keene, NH). The mRNA for IL-12Rβ2 was probed with a purified fragment of murine IL-12Rβ2 subunit cDNA generated by a PCR reaction. Briefly, RNA (1 μg) was synthesized into cDNA by reverse transcription using SUPERSCRIPT II (Life Technologies, Gaithersburg, MD) in the presence of random oligo(dT)15-18 primer. cDNA (1 μl) was amplified by PCR with PCR beads (Pharabiotype, Piscataway, NJ) in the presence of specific primers for IL-12Rβ2. 5′-CTGCACACCACCTACATTACA-3′, CAGTTGGCTTTGCCCTGTGG. The β actin primer was obtained from Clontech (Palo Alto, CA). The PCR product was purified before used as the probe for Northern blot analysis.

Results

Differential effects of APL on proliferative responses and cytokine production

Ac-11 of MBP is the major encephalitogenic epitope in H-2b mice (29–31). It has previously been shown that P at position six and Q at position three are the primary and secondary TCR interaction residues, respectively, while K at position four and R at position five are the critical amino acids responsible for interaction with I-Aκ (Table I). Analysis of the reactivity profile of a T cell hybridoma specific for Ac-11 demonstrated that certain amino acid substitutions at position three lead to the generation of peptide analogues with different affinities for the TCR. These peptide analogues were weak agonist peptides that induced diminished IL-2 production in the presence of the weak agonists. Taken together, some of the peptides induced as much IL-3 as the WT peptide tested both 3M and 3Y were equivalent to WT (Fig. 1B); similarly, the variant peptides induced significantly less T cell proliferation than the WT peptide, although at the highest concentration tested both 3M and 3Y were equivalent to WT (Fig. 1A); similarly, the variant peptides were much less efficient than the WT peptide in the induction of IL-3 production, but again at the highest concentration tested some of the peptides induced as much IL-3 as the WT (Fig. 1B). In contrast, none of the variant peptides were capable of inducing significant amounts of IFN-γ (Fig. 1C).

Table I. The amino acid sequence of Ac-11

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*The concentrations of peptide required for induction of 50% maximum IL-2 production by a T cell hybridoma were (29): WT, 20 μg/ml; 3M, 20 μg/ml; 3H, 100 μg/ml; 3F, 3Y, 500 μg/ml; 4Y, 0.01 μg/ml.
Substitution of 4K of Ac1-11 with Y creates a peptide analogue that binds to I-A^u with 1500 times higher affinity than does the WT peptide. All of the doubly variant peptides (3M4Y, 3H4Y, 3F4Y, and 3Y4Y) induced T cell proliferation, IFN-\(\gamma\), and IL-3 production at an enhanced level compared with the WT peptide (Fig. 1, D to F). The peptide analogue with single amino acid substitution at the MHC anchor residue only (4Y) also induced an enhanced level of proliferation and cytokine production compared with the WT peptide ligand (data not shown). The differences observed between 4Y and the doubly-substituted peptides (3M4Y, 3H4Y, 3F4Y, and 3Y4Y) were not significant. Therefore, the enhanced MHC binding of the 4K > 4Y substitution converts a weak agonist peptide to a peptide analogue with superagonist properties. IL-4 was not detected in any of the supernatants of these cultures (data not shown).

**Differential induction of CD80/CD86 expression on APC and CD40L expression on T cells by native and variant peptides**

The induction of T cell proliferation and cytokine production are critically dependent on costimulatory signals delivered to the Ag-specific T cell by the APC. To determine whether the decreased T cell proliferation and cytokine production induced by the weak agonist peptides were due to their differential capacity to induce expression of costimulatory activities on APC, we analyzed the expression of CD86 on B220^+ splenic B cells in cultures of transgenic spleen cells stimulated with either WT peptide or APL. Stimulation with WT peptide resulted in marked up-regulation of CD86 expression; in contrast, the weak agonists (3M, 3H, 3F, and 3Y) induced only a minimal expression of CD86 (Fig. 2A). Similar results were observed when the expression of CD80 was analyzed, although the maximum number of cells that expressed CD80 when the WT peptide was tested was much lower than the percentage of CD86-expressing cells (data not shown). The superagonist 4K > 4Y peptide resulted in enhanced expression of CD86 on B220^+ spleen cells. As was seen in the proliferation and cytokine production assays, the substitution of K by Y at position four resulted in conversion of the weak agonists to superagonists in terms of their capacity to induce CD40L expression (Fig. 2B).

![FIGURE 1. Proliferative responses and cytokine production by CD4^+ T cells from the TCR transgenic mice stimulated with WT peptide or APL. Spleen and lymph node cells from TCR transgenic mice were stimulated with different concentrations of WT peptide A to F (■) or APL A to C, 3H (●), 3F (○), 3Y (▲), 3M (□); D to F, 3M4Y (■), 3H4Y (●), 3F4Y (○), 3Y4Y (▲). T cell proliferation was assayed after 48 h of culture; IL-2 was assayed after 24 h of culture, while IFN-\(\gamma\) and IL-3 were measured after 48 h of culture.](http://www.jimmunol.org/)

![FIGURE 2. Induction of CD40L (B) and CD86 (A) expression on CD4^+ T cells and B220^+ splenic B cells by WT peptide or APL. Spleen cells (5 × 10^6/ml) from the transgenic mice were cultured with either WT peptide or APL at Ag concentration of 10 \(\mu\)M for 18 h; the cells were then harvested and expression of the CD40L was analyzed on the gated CD4^+ cells and the expression of CD86 was analyzed on gated B220^+ population. The percentage positive in the gated population is shown.](http://www.jimmunol.org/)
Anti-CD28 costimulation can synergize with weak agonists to enhance IL-2 production and proliferation, but not IFN-γ production

The results obtained thus far raise the possibility that the failure of the weak agonist peptides to induce expression of the CD40L is responsible for all of the defects in the subsequent T cell activation cascade (CD80/CD86 expression, proliferation, and cytokine production). It was therefore of interest to determine whether the addition of anti-CD28 would reverse the defective stimulatory capacity of these APL. Engagement of CD28 by a stimulatory mAb should lead to both enhanced cytokine production and enhanced expression of the CD40L (32), which should further stimulate expression of costimulatory molecules and production of APC-derived cytokines such as IL-12. Indeed, addition of soluble anti-CD28 to the cultures of spleen cells from the transgenic mice resulted in a significantly enhanced proliferative response and IL-2 production, both in the presence of WT peptide and the weak agonist APL (Fig. 3). Although anti-CD28 enhanced the amount of IL-2 produced by the CD4+ T cells stimulated with the weak agonist APL, IL-2 production was still less than that seen when the CD4+ T cells were stimulated with WT peptide, and the effect of anti-CD28 was most prominent at high concentrations of the APL. Anti-CD28 shifted the dose-response curves for both the WT and weak agonist peptides so that a lower concentration of peptides was required in both cases. In contrast to the effects of CD28 ligation on proliferation and IL-2 production, CD28 ligation only slightly increased IFN-γ production by the CD4+ T cells stimulated with either the WT peptide or the weak agonist APL at all Ag concentrations tested (Fig. 3, E, F).

These results strongly suggested that the capacity of anti-CD28 to deliver a costimulatory signal for some components (CD40L), but not others (IL-2 production), of the T cell activation cascade might be inherently limited by the strength of the antigenic signal delivered to the TCR. We therefore directly examined the effects of CD28 ligation on the induction of the CD40L in the presence of WT and weak agonist peptides. Although high levels of CD40L expression were induced by the WT peptide, no augmentation of CD40L expression was seen when the cultures were supplemented with anti-CD28. Furthermore, only low levels of CD40L expression were seen when the cultures were stimulated with the weak agonists 3F and 3Y and no augmentation of CD40L expression was seen when anti-CD28 was added (Fig. 4).

Ag-specific IFN-γ production by CD4+ T cells from transgenic mice is IL-12 dependent

The failure of anti-CD28 to augment IFN-γ production by the WT peptide or the weak agonist peptides strongly suggested that maximal engagement of CD28 was not sufficient to enhance CD40L expression to a degree required for engagement of CD40 with resultant IL-12 production. To directly determine the contribution of IL-12 to this system, CD4+ T cells were stimulated with either WT, weak agonist, or superagonist peptides in the presence or absence of IL-12 or anti-IL-12. Addition of a neutralizing mAb to IL-12 completely abolished the capacity of the superagonist series of peptides to induce IFN-γ production by the transgenic T cells (Fig. 5, A vs B). Thus, the capacity of the superagonist to induce IFN-γ production was completely IL-12 dependent. Similarly, the defective ability of the weak agonist peptides to induce IFN-γ production was markedly augmented by the addition of exogenous IL-12. Most importantly, the effects of exogenous IL-12 in the induction of IFN-γ production were so potent that 1,000 to 10,000-fold lower concentrations of WT peptide were needed to reach the level of IFN-γ production seen in the presence of WT peptide in
of the weak agonist peptides to stimulate IFN-γ production. To directly determine whether the observed defect in the capacity of APC to activate T cells is correlated with the strength of the TCR signal, T cells from transgenic mice were stimulated with either WT peptide (■), 3M4Y (◆), 3H4Y (○), or 3Y4Y (▲) in the presence of normal rat IgG (▲) or anti-IL-12 (10 μg/ml) (▼). C and D: Purified CD4+ T cells were stimulated with WT peptide (■), 3M (◆), 3H (○), 3F (▲), 3Y (▲) in the absence (C) or presence of murine IL-12 (10 ng/ml) (D). IFN-γ production after 48 h was analyzed by ELISA.

The absence of exogenous IL-12 (Fig. 5 C and D). Thus, IL-12, but not anti-CD28, can synergize with weak TCR signaling to induce maximal IFN-γ production.

**IL-12 production by APC is correlated with the strength of the TCR signal**

To directly determine whether the observed defect in the capacity of the weak agonist peptides to stimulate IFN-γ production by the CD4+ T cells was secondary to defective induction of IL-12 production by APC, we analyzed IL-12 production at the protein level in cultures of stimulated spleen cells from the TCR transgenic mice. An Ag dose-dependent production of IL-12 p40 was detected in the supernatants of cultures stimulated with any of the four weak agonist peptides (Fig. 6). IL-12 p40 could also be detected in supernatants of cultures stimulated with the doubly substituted superagonist peptides (3M4Y, 3H4Y, 3F4Y, and 3Y4Y).

**Discussion**

In the present study, we used a population of nonactivated spleen cells from mice that expressed a transgenic TCR specific for MBP Ac1-11 and a set of peptide analogues that display either enhanced or decreased affinities for TCR/MHC to address the question whether APL-induced signaling through the TCR can regulate the capability of APC to activate T cells. We examined the expression of costimulatory molecules on and the secretion of IL-12 by APC in unseparated spleen populations in order to avoid any unwarranted effects of in vitro manipulations (adhesion, radiation, etc.) on APC function. It has previously been shown that substitutions at position 3 (3F, 3M, 3H, 3Y) of MBP Ac1-11 generated weak agonist peptides in terms of their capability of activating cytokine production by an Ac1-11-specific T cell hybridoma. We also observed that these weak agonists were very poor activators of proliferation and IL-2/IL-3 production by freshly explanted T cells from the anti-MBP TCR transgenics. However, at higher concentrations of peptides, some of these APL could activate proliferation and IL-3 production to the same extent as the WT peptide. In contrast, very little IFN-γ production was seen at even the highest concentration of weak agonist peptide tested, while significant induction of IFN-γ production was seen in the presence of the WT peptide. It has previously been shown that the MBP Ac1-11 (4K > 4Y) substitution resulted in a 1000-fold or greater enhancement of binding to MHC class II and that this peptide functioned as a
superagonist. Indeed, all of the doubly substituted peptides tested (3F4Y, 3M4Y, 3H4Y, and 3Y4Y) as well as 4Y itself had superagonist properties in terms of induction of T cell proliferation, IL-2/IL-3 production, and IFN-γ production.

A striking correlation was seen between the failure of the weak agonist peptides to activate T cells and their failure to induce expression of CD80/CD86 on APCs and to induce the secretion of IL-12. In addition, all of the weak agonists were poor inducers of CD40L expression on the responder T cells. Again, enhancement of MHC binding by introduction of the 4K > 4Y substitution restored the capacity of the weak agonists to induce CD80/CD86 expression, IL-12 secretion by the APC, and high levels of CD40L expression on the responder T cells. Although we have had difficulties detecting IL-12 heterodimer by ELISA, the production of IL-12 p40 did correlate with the induction of IFN-γ production; furthermore, our ability to inhibit IFN-γ production induced by high concentrations of the superagonist peptides with anti-IL-12 illustrates that IFN-γ production is completely dependent on IL-12 in this transgenic model.

Over the past several years, considerable experimental data have been accumulated that have suggested that induction of CD40L expression on T cells is the critical step in the cascade of events that are involved in T cell-APC interactions. Although the CD80/CD86 family of costimulatory molecules can be induced on APC by bacterial products such as LPS, T cell-derived cytokines (IL-4 and IFN-γ) and engagement of peptide MHC class II complexes, many studies have suggested that the major initiator of the up-regulation of costimulation is the CD40L/CD40 interaction. Similarly, although the production of IL-12 by dendritic cells, macrophages, and neutrophils can be stimulated by intracellular pathogens via a T cell-independent pathway (34, 35), studies in CD40L- and/or CD40-deficient mice strongly suggest that the major physiological inducer of IL-12 production is again the CD40L/CD40 interaction (25, 36, 37). It has also been claimed that induction of expression of the CD40L was not dependent on costimulation via CD28 as the induction of CD40L expression on transgenic T cells was not inhibited by CTLA4Ig or by anti-CD80/CD86 mAbs (38). Furthermore, the induction of the CD40L by plate-bound anti-CD3 at low concentration was not enhanced by the presence of CD80-transfected L cells or the presence of anti-CD28. In an Ag-induced model with transgenic T cells, the low level of CD40L expression induced by CD80/ICAM-negative L cells could also not be enhanced by the addition of unpulsed CD80/ICAM-positive L cells or anti-CD28 (39). These results differ from our previous report (32), which demonstrated that CD40L expression could be augmented by CD80/CD28 signaling. However, it is difficult to compare these studies as the latter experiments were performed with L cells rather than with normal spleen cells as APC. It is possible that the signal required for enhancement of CD40L induction by CD28 saturates at a lower level than the signal for regulating the level of CD40L expression and that the most important signal for regulating the level of CD40L is through the TCR.

The major conclusions drawn from these studies were that expression of the CD40L is controlled by the strength of the signal delivered via the TCR and that all of the augmenting role that has been observed for accessory molecules such as CD80 and ICAM-1 was secondary to enhancement of cell-cell contact and delivery of signals via the TCR. Our results on the activation of the MBP Ac1-11-specific transgenic T cells by APL are consistent with this paradigm. Activation by the weak APL resulted in a poor delivery of a signal via the TCR, while the 4Y substitution, which enhanced MHC binding, also enhanced delivery of the TCR signal and CD40L expression. Increasing costimulation by the addition of anti-CD28 only enhanced IL-2 production and T cell proliferation, and also failed to produce enhancement of CD40L expression (Figs. 3 and 4). One difficulty encountered in the interpretation of all of these studies is that they have primarily examined the effects of CD28 signaling on IL-2 production and/or T cell proliferation. One advantage of the model system we have used is that the transgenic T cells did not have to be primed for several days to differentiate into Th1-like IFN-γ producers and we could simultaneously assay the costimulatory requirements for proliferation and IFN-γ production. IFN-γ was readily detected within 48 h after stimulation in culture supernatants and restimulation was not required. The difference between these findings and other studies may be due to our use of the normal APC population in unseparated spleen as the source of endogenous IL-12. We have found that irradiation significantly impairs the production of IL-12 by T-depleted spleen populations (data not shown). It remains possible that some of the IFN-γ may have been produced by memory T cells, but previous studies have demonstrated that activated transgene-positive cells could not be detected in these animals (26). Although the failure of the weak agonists to induce IL-12 production and IFN-γ production again correlated with their weak effects on induction of the CD40L, the addition of exogenous IL-12 readily restored their ability to induce IFN-γ production in a dose-dependent manner. Most importantly, the amount of IFN-γ induced by the APL in the presence of IL-12 was 5- to 10-fold greater than that seen when the same T cells were stimulated by the WT peptide in the absence of IL-12 (Fig. 5).

Several studies have shown that resting T cells do not respond to exogenous IL-12 either by the production of IFN-γ or by proliferation and that activation via the TCR is required to induce up-regulation of IL-12R expression. Our ability to reconstitute IFN-γ production in the presence of the weak agonists suggests that relatively low affinity TCR signals were needed to induce functional IL-12R expression. IL-12Rβ2 chain mRNA was readily detected when the transgenic T cells were stimulated with the WT peptide, but barely detectable when the 3Q > 3F APL was used; addition of IL-12, but not anti-CD28, resulted in marked up-regulation of IL-12Rβ2 chain mRNA. In contrast, a much higher threshold of TCR signaling was required for induction of CD40L expression, CD40L/CD40 interaction, and IL-12 production. TCR signaling can therefore regulate IFN-γ production by CD4+ T cells, both at the level of IFN-γ gene transcription and at the level of IL-12R expression, the latter determining responsiveness to IL-12. IL-12 then plays a major role in up-regulating its own receptor.

There are a number of important implications of our results with regard to the role of the strength of TCR signaling in directing Th1/Th2 differentiation. Pearson et al. (9) have analyzed the responsiveness of T cells from an anti-MBP Ac1-11 TCR transgenic animal to activation by the superagonist, MBP Ac1-11 4Y. In contrast to our findings, they found that 4Y preferentially induced the Th2 cytokines IL-4 and IL-10 in vivo and in vitro. The conclusion drawn from this study was that the enhanced affinity of the Ac1-11 4Y for the MHC preferentially enhanced its ability to stimulate Th2 responses, perhaps secondary to the resultant strong TCR-mediated up-regulation of CD86. However, interpretation of this study is complicated by the property of the 4Y peptide to induce apoptosis in vivo with perhaps preferential survival of Th2 cells. Similarly, in contrast to our studies, which demonstrated that 4Y was a potent inducer of IFN-γ in vitro, Pearson et al. (9) demonstrated that, while the WT peptide readily induced IFN-γ, no IFN-γ was produced in response to 4Y. However, these studies were performed by stimulating the transgenic T cells for 10 days in vitro prior to restimulation and assay; in our hands, significant cell death is observed when the transgenic T cells are cultured with
4Y under these conditions and the production of Th2-like cytokines after prolonged culture may be secondary to differential survival of a subpopulation of the transgenic cells. We have failed to observe IL-4 or IL-10 production by freshly explanted transgenic T cells in our short term assays. Our studies are much more compatible with some of the results of Bottomly’s group (8, 40), which have also used APL with weak agonist properties and shown that such APL stimulated the priming of both IFN-γ and IL-4 producers, while the WT peptide only primed for IFN-γ production. Furthermore, the priming for IL-4 production by the weak agonist peptides could be augmented by anti-CD28 (40). While one might conclude from these studies that the high density of Ag-MHC class II on APC favors Th1 responses, while low density favors Th2 responses, one important factor that is very apparent from our results is that the production of IFN-γ occurs very rapidly in primary responses upon stimulation with superagonist peptides like 4Y, while production of IL-4 is observed only upon priming and re-stimulation. As even low concentrations of IFN-γ may block priming of IL-4 producers (8), the effects of high concentrations of Ag on Th2 priming can be assayed only under conditions in which production of IFN-γ is inhibited by inclusion of anti-IFN-γ and anti-IL-12 into the cultures in the absence of exogenous IL-4. Such studies using the panel of strong and weak agonists described in this report are now in progress.

Lastly, there are a number of important implications of our findings vis-à-vis the activation of autoreactive T cells in vivo. It is widely accepted that one mechanism by which T cells escape tolerance induction and deletion in the thymus is that the avidity of MHC-peptide TCR complex is low. Indeed, the low affinity of Ac1-11 for MHC may explain why T cells specific for this epitope escape tolerance induction (41, 42). The critical question that remains is how such autoreactive T cells become primed in vivo to differentiate into pathogenic Th1 effectors? One possibility is that, under certain pathological conditions in which professional or non-professional APC are activated by microbial products or T cell-generated cytokines to express high levels of CD80/CD86, these APC may provide sufficient costimulation to induce the naive autoreactive T cells to differentiate into Th1 effectors. However, the delivery of costimulatory signals via CD28 is efficiently achieved only when the APC express both the stimulatory peptide and CD80/CD86 (43, 44). Second, our results clearly demonstrate that even when high concentrations of weak agonists are used in vitro, direct engagement of CD28 by agonist Abs is insufficient to promote Th1 differentiation. There appears to be a critical threshold of TCR signaling required for differentiation along the Th1 lineage, and even maximum delivery of costimulation cannot overcome this barrier. On the other hand, our data strongly implicate IL-12 as the critical “danger signal” operative in the activation of low affinity autoreactive T cells. Exogenous IL-12 readily induced IFN-γ production in an Ag-dependent fashion with all the weak agonist APL. It is likely that IL-12 can be effective in this capacity when secreted in trans by cells that do not express the target peptide or costimulatory molecules. We have recently demonstrated that exposure of MBP-specific T cells to LPS, to highly-purified bacterial DNA, or to CpG containing oligonucleotides resulted in a conversion of these from a quiescent state to autoimmune disease effector Th1 cells by an IL-12-dependent pathway (45). Thus, the production of IL-12 in the immediate environment of low affinity autoantigen-specific T cells and their target autoantigen may be the major driving force for the differentiation of pathogenic autoimmune effector cells.

References


