

Altered Peptide Ligands Can Control CD4 T Lymphocyte Differentiation In Vivo

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Summary

Antigen priming of naive CD4 T cells can generate effector CD4 T cells that produce interleukin 4 (T helper [Th]2-like) or interferon- γ (Th1-like). Using a system in which priming leads to responses dominated by one or the other of these cell types, we show that varying either the antigenic peptide or the major histocompatibility complex class II molecule can determine whether Th1-like or Th2-like responses are obtained. Our results show that peptide/major histocompatibility complex class II complexes that interact strongly with the T cell receptor favor generation of Th1-like cells, while those that bind weakly favor priming of Th2-like T cells. Thus, signals from the T cell receptor can influence the differentiation of CD4 T cells into specific types of effector cells.

The type of effector CD4 T lymphocyte activated during an adaptive immune response determines the efficacy of the response in protective immunity to pathogens. This, in turn, is controlled by selective generation of distinct subsets of effector CD4 T cells (1, 2) known as Th1 cells, which can activate macrophages and release, upon activation, the cytokines IFN- γ and lymphotoxin (3–5), and Th2 cells, which induce B cells to secrete IgG1 and IgE antibodies and produce the cytokines IL-4 and IL-5 (2, 6, 7). While little is known about the mechanisms by which selective differentiation of naive CD4 T cells into Th1 or Th2 effector cells is achieved, cytokines produced in the early phases of some infections clearly have an impact on this process (8–16). In this report, we show that the selective generation of a CD4 T cell functional class can also be influenced by the nature of the antigen itself. Specifically, we show that subtle modifications in a peptide used for primary immunization in vivo can lead to selective generation of Th1 or Th2 cells. Thus, signals delivered via the TCR can influence CD4 T cell differentiation.

In earlier studies, we have shown that mice primed with human collagen IV (huColIV) developed either IL-4-secreting Th2 cells or IFN- γ -secreting Th1 cells, depending on their genotype at MHC class II (17, 18). Strains of mice having the MHC class II molecule I-A^b produced serum antibody in response to huColIV, but did not prime T cells which would proliferate in a secondary response. This was in contrast to mice expressing I-A^s, whose T cells proliferated upon rechallenge, but did not induce an antibody response. This difference was due to selective activation of Th2-like CD4 T cells in H-2^b mice and Th1-like CD4 T cells in H-2^s

mice (18). Both mouse strains responded to a single 12-amino acid peptide within the huColIV molecule (19) by generating their characteristic set of CD4 T cells (reviewed in Table 1). As the functional class of the response was influenced by the genotype of the MHC class II molecule, which presents peptides to CD4 TCR, we proposed that the ability of MHC class II genotype to control CD4 T cell differentiation could be due to differences in the repertoire of T cells responding to the antigen, or to differences in peptide/MHC class II structure and/or abundance (20). The former seemed unlikely, because it would link MHC allele specificity to function and is now known to be wrong, since mice transgenic for a known TCR can generate responses dominated by either Th1- or Th2-like cells that use the transgene-encoded receptor (21, 22). We have, therefore, focussed upon the latter possibility, both by measuring the strength of binding of the immunodominant peptide of huColIV to purified MHC class II molecules and by examining the effect of peptide modification on CD4 T cell subset differentiation. Our results show that the nature of the ligand for the TCR can control the differentiation of naive CD4 T cells into committed effector cells.

Materials and Methods

Animals. A.SW/SnJ and A.BY/SnJ mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and used at 6–12 wk of age.

Antigens. Synthetic peptides coding for residues 675–686 of the alpha chain of huColIV were prepared by solid phase chemistry on a peptide synthesizer (model 430-A; Applied Biosystems, Inc., Foster City, CA), and were purified by HPLC.

CD4 T Cell Proliferation Assays. CD4 T cells were isolated and cultured, as previously described (18), from mice immunized 10 d previously.

Peptide Binding Assays. I-A^s and I-A^b molecules were purified, as previously described (23, 24). The relative avidity was measured by competing binding of radiolabeled reference peptide (ROIV), to purified I-A^s or I-A^b.

Cytokine mRNA Expression. cDNA was amplified with 35 cycles of PCR (94° for 1 min, 60° for 2 min, 72° for 3 min), using Taq polymerase (Promega Corp., Madison, WI). PCR products were run on 2% agarose gel by electrophoresis. Control cDNA was prepared from the activated Th1 clone, C19, which secretes IFN- γ , but not IL-4, upon activation; the activated Th2 clone, D10, which secretes IL-4, but not IFN- γ , upon activation; and CD4 T cells from KLH-primed mice. Primers: IFN- γ (sense) 5'-TGA-ACGCTACACACTGCATCTTGG and (antisense) 5'-CGACTCCTTTTCCGCTTCCTGAG; IL-4 (sense) 5'-ATGGGTCTCAAC-CCCCAGCTAGT and (antisense) 5'-GCTCTTTAGGCTTTC-CAGGAAGTC; CD3 δ (sense) 5'-GCAGCTGGCAAAGGTGGT-GTCTTC and (antisense) 5'-TATGGCACTTGTAGAAACCTCCAT.

Cytokine Bioassays. For IL-2 measurement, serial dilutions of supernatant from cultures described above were added to 10,000 CTL line indicator cells in the presence or absence of anti-IL-2 antibody. Cultures were pulsed after 48 h with 1 μ Ci [³H]dThR and harvested 18–24 h later. Standard plates were set up with several titrations of recombinant murine IL-2, and units were calculated from standard curves with one unit being defined as the amount of IL-2 that gave half-maximal proliferation. For IFN- γ measurement, dilutions of supernatant were incubated with 5,000 WEHI-279 cells in the presence or absence of anti-IFN- γ antibodies. Proliferation was assessed after 48 h, with 1 U of IFN- γ defined as the concentration which gave half-maximal inhibition of WEHI-279 proliferation, calculated from the standard curve obtained with mouse IFN- γ standard (Genzyme Corp., Cambridge, MA). In all assays, inhibition of proliferation was blocked by anti-IFN- γ .

Results

Relative Binding Avidity of huColIV Peptides to MHC Class II. The binding of the immunodominant huColIV peptide to MHC class II I-A^s and I-A^b molecules was determined using affinity-purified MHC class II molecules extracted from B lymphoma cells (23, 24). The same reference peptide (ROIV) was used as a labeled probe for binding to both I-A^b and I-A^s, and its ability as a cold peptide inhibitor was used to standardize binding to the two different MHC class II molecules. ROIV bound both I-A^s and I-A^b well (Fig. 1) with IC₅₀ in the 20–40 nM range. Other controls in the experiments were the I-A^s-restricted epitope myelin basic protein 81–100 (25), which bound I-A^s as well as the ROIV peptide, but bound with far less affinity for I-A^b, and the I-A^d-restricted peptide staphylococcal nuclease 91–100 (26), which bound both I-A^b and I-A^s with IC₅₀ in the 0.5–1.0 μ M range. Using this assay, it can be seen (Fig. 1) that the immunodominant peptide of huColIV (wild-type) binds to I-A^s 2 \times 10⁴-fold more avidly than it binds to I-A^b. These data are consistent with the hypothesis that presentation of different densities of huColIV peptide to T cells can lead to selective priming of Th1 or Th2, as observed in earlier studies

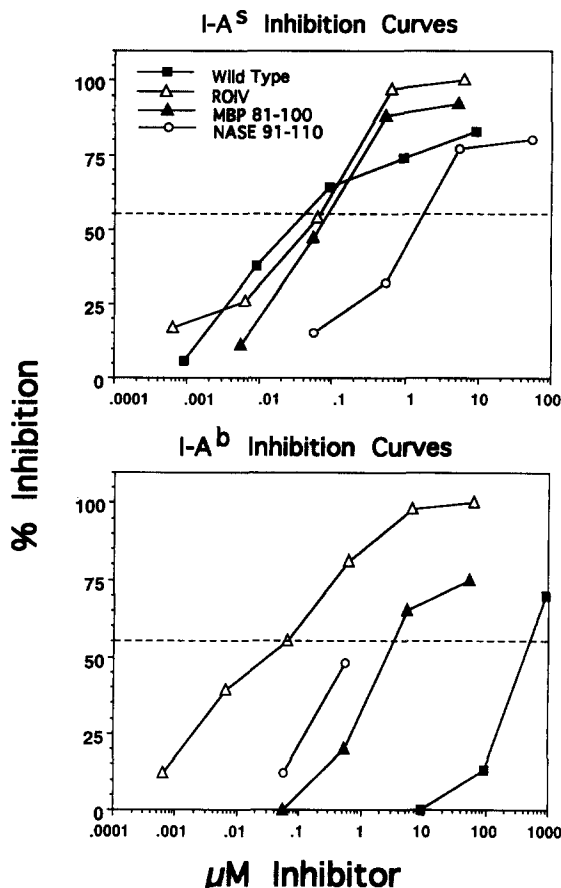


Figure 1. Relative avidity of I-A^s- and I-A^b-binding peptides. The relative avidity was measured by competing binding of ROIV to purified I-A^s or I-A^b. The amount (μ M) of each peptide (wild-type, α 2[675–686] huColIV; MBP, myelin basic protein peptide 81–100; Nase, Staphylococcus nuclease 91–110; ROIV, superbinder used as the reference peptide because it binds both I-A^s and I-A^b well) required to inhibit the binding of the ROIV (23, 24) to either I-A^s (top) or I-A^b (bottom) is shown.

(20). When mice of I-A^b genotype were immunized with progressively higher doses of huColIV wild-type peptide, we did indeed observe a shift from Th2 to Th1. Likewise, increasing the dose of peptide injected into I-A^s eventually shut down the production of Th1 cytokines. However, we did not observe a dose of huColIV peptide at which the H-2^s mice developed CD4 T cells making Th2 cytokines. Thus, it appears that dose of MHC/peptide by itself cannot control Th1 versus Th2 differentiation.

Mutant ColIV Peptides that Elicit IL4, Rather than IFN- γ , responses from CD4 Cells in I-A^s Mice. The problem with this type of analysis is that I-A^b and I-A^s molecules differ by many amino acid residues, and the responding T cells have developed under different intrathymic selective conditions. Thus, it is difficult to make direct comparisons of T cell responses between strains, even if they differ only at the MHC. To circumvent this difficulty, we have prepared mutants of the huColIV immunodominant peptide that differ in their binding to I-A^s or I-A^b, but nevertheless could activate a

panel of T cell hybrids, specific for the wild-type peptide bound to the appropriate MHC class II molecules, as shown in Figs. 2 and 3. These peptides were then tested for their ability to prime Th1- or Th2-like CD4 T cells in a single strain of mouse.

Two mutant peptides, $\alpha 2\text{Glu}(5)$ and $\alpha 2\text{Ala}(11)$, which required higher concentrations than the wild-type peptide to activate a panel of I-A^s-restricted, huColIV-specific T cell hybridomas were identified (Fig. 2 A). These peptides also bound to I-A^s somewhat less well than the wild-type peptide. Purified CD4 T cells from mice primed with $\alpha 2\text{Glu}(5)$, $\alpha 2\text{Ala}(11)$, or the wild-type peptide were analyzed for their ability to proliferate to the wild-type peptide in vitro, presented by antigen-presenting cells from I-A^s mice. As can be seen in Fig. 2 B, CD4 T cells from $\alpha 2\text{Ala}(11)$ and $\alpha 2\text{Glu}(5)$ showed little, if any, proliferative response in comparison to the response of CD4 T cells from H-2^s mice primed with the wild-type peptide. Messenger RNA (mRNA) was also isolated from the CD4 T cells in these secondary cultures, reverse transcribed into cDNA, and assayed by cytokine-specific polymerase chain reaction for cytokine-specific mRNA. As shown in Fig. 2 C, mRNA specific for IFN- γ , but not IL-4, was produced by CD4 T cells from H-2^s mice that were primed with the wild-type peptide, but not from CD4 T cells primed with the mutant peptides, $\alpha 2\text{Ala}(11)$ and $\alpha 2\text{Glu}(5)$, and restimulated with the wild-type peptide. By contrast, IL-4 mRNA appeared only in CD4 T cells from H-2^s mice primed with $\alpha 2\text{Ala}(11)$ and $\alpha 2\text{Glu}(5)$ and restimulated by

the wild-type peptide. Thus, a decrease in the ability of the MHC class II/peptide complex to activate a group of huColIV-specific T cell hybridomas led to a change in the differentiation of naive CD4 T cells upon priming, as reflected in a change in cytokines produced from IFN- γ to IL-4. This change correlates much better with the differences in the ability to activate a panel of hybridomas specific for wild-type huColIV than with binding to I-A^s.

Mutant huColIV Peptides that Stimulate I-A^b-specific Hybrids 100-fold Better Can Prime Th1-like CD4 T Cells in H-2^b Mice. To analyze CD4 T cell differentiation in H-2^b mice, one peptide with an Ala substitution at position 10, $\alpha 2\text{Ala}(10)$, was identified that had an 80-fold increased ability to activate a panel of H-2^b-restricted huColIV-specific T cell hybridomas (Fig. 3 A). Its ability to bind I-A^b was not distinct from that of the wild-type huColIV peptide. When $\alpha 2\text{Ala}(10)$ was used to prime H-2^b mice, the CD4 T cells revealed an excellent recall proliferative response to the wild-type peptide presented by H-2^b antigen-presenting cells, whereas priming with the wild-type peptide did not (Fig. 3 B). Analysis of cytokine mRNA expression of the CD4 T cells restimulated in vitro with wild-type peptide demonstrated that CD4 T cells from mice primed with the wild-type peptide made IL-4, but not IFN- γ , mRNA, as shown previously (Table 1), but CD4 T cells from mice primed with the mutant peptide $\alpha 2\text{Ala}(10)$ no longer expressed IL-4 mRNA, but IFN- γ mRNA (Fig. 3 C). Thus, in this case

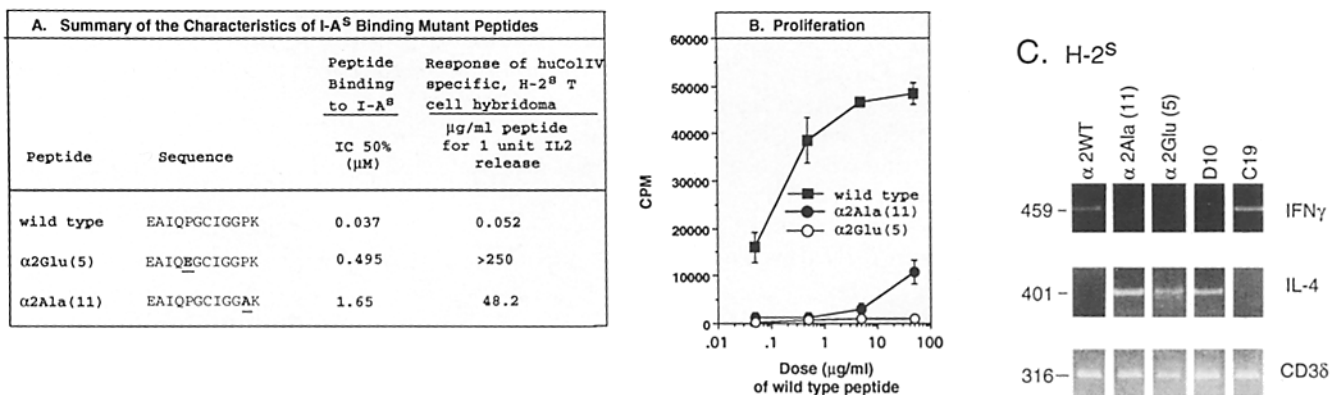


Figure 2. Immunization with mutant peptides changes the type of effector CD4 T cell generated during priming of H-2^s mice. (A) Summary of the I-A^s mutant peptides. Peptide binding is determined by the amount of peptide (μM) required to inhibit the binding of ROIV to I-A^s by 50% (IC₅₀). The response of the huColIV-specific, I-A^s-restricted T cell hybridoma is determined by culturing 2×10^5 T hybridoma cells and 2×10^5 irradiated T-depleted spleen cells with varying concentrations of peptide. After 24 h, supernatants were collected and analyzed for presence of IL-2, using the CTL line IL-2 bioassay. The peptide concentration ($\mu\text{g/ml}$) required for the release of 1 U IL-2/culture is used to compare T hybridoma activation by wild-type and mutant peptides. The data represent one of five individual experiments giving the same results. (B) The proliferative response of CD4 T cells from wild-type and mutant peptide-primed H-2^s (A.SW) mice. CD4 T cells were isolated, as described previously (18), from mice immunized 10 d previously with $2 \mu\text{g}$. 2×10^5 CD4 T cells/well were cultured with the wild-type peptide (0.05–50 $\mu\text{g/ml}$) and 2×10^5 T-depleted splenocytes (mitomycin C-treated) as antigen presenting cells. Cultures were incubated for 72 h, followed by an overnight pulse with $1 \mu\text{Ci/well}$ [³H]thymidine. The background cpm without antigen was < 1,500 and has been subtracted from the results. Plotted values represent mean \pm SD of triplicate cultures. All experiments were repeated at least three times. (C) Cytokine mRNA expression upon priming H-2^s (A.SW) mice with mutant and wild-type peptides. CD4 T cells from mutant and wild-type peptide-primed H-2^s (A.SW) mice (primed with $2 \mu\text{g}$ peptide/mouse) were isolated from the local lymph nodes and were cultured in 24-well plates at 2×10^6 cells/well in the presence of 10 $\mu\text{g/ml}$ wild-type peptide and 1×10^6 syngeneic T-depleted splenocytes (mitomycin C-treated) from normal, nonimmunized, syngeneic mice. After 48 h, mRNA was made (Micro-Fast Track; Invitrogen, San Diego, CA); cDNA from 2×10^4 cells was prepared and used for each PCR and was equalized using CD3 β as a control. These sequence data are available from GenBank/EMBL/DBJ under accession numbers K00083 (IFN- γ), X05064 (IL-4), and X02339 (CD3 β).

| A. Summary of the Characteristics of I-A ^b Binding Mutant Peptides | | | |
|---|--------------|-------------------------------------|---|
| Peptide | Sequence | Peptide Binding to I-A ^b | Response of huColIV specific, H-2 ^b T cell hybridoma |
| | | IC 50% (μM) | μg/ml peptide for 1 unit IL2 release |
| wild type | EAIQPGCIGGPK | 615 | 39 |
| α2Ala(10) | EAIQPGCIAGPK | 451 | 0.5 |

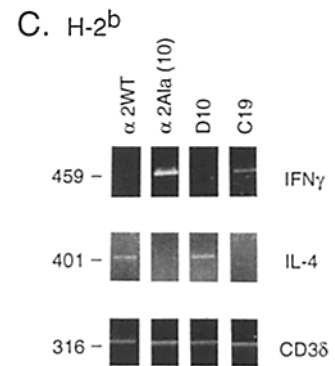
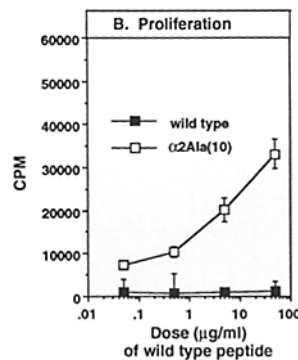


Figure 3. Immunization with mutant peptides changes the type of effector CD4 T cells generated during priming in H-2^b mice. (A) Summary of the I-A^b-binding peptides. Peptide binding is determined as described in the Fig. 2 legend and Materials and Methods. The response of the huColIV-specific, I-A^b-restricted hybridoma was determined as described in the Fig. 2 legend. (B) The proliferative response of CD4 T cells from wild-type and mutant peptide-primed H-2^b (A.BY) mice (see Fig. 2 legend). (C) Cytokine mRNA expression upon priming of H-2^b (A.BY) mice with mutant and wild-type peptides (see Fig. 2 legend and Materials and Methods).

as well, the ability of an huColIV peptide to interact with MHC and the TCR gives rise to a phenotypically distinct response.

Priming Dose of Peptide Regulates the Type of Effector CD4 T Cells Response in H-2^b. Given that our initial findings showed that increasing the priming dose of huColIV wild-type peptide in H-2^b leads to a shift from Th2 to Th1 cells (20), and given that the mutant peptide α2Ala(11) could prime Th2 cells in H-2^b mice, we tested whether increasing the priming dose of α2Ala(11) would lead to a shift in priming from Th2 to Th1 cells in H-2^b mice as well. CD4 T cells from mice primed with either 2 μg or 50 μg of wild-type or mutant peptides were analyzed for IL-4 or IFN-γ production in vitro upon restimulation with the wild-type peptide. As can be seen in Fig. 4, mRNA specific for IFN-γ and IFN-γ protein were observed after restimulation when the CD4 T cells were derived from wild-type or α2Ala(11) primed mice. No IL-4 was observed from these same primed CD4 T cells. However, when CD4 T cells were derived from mice

primed with 2 μg of peptide, the CD4 T cells from α2Ala(11)-primed mice shifted from IFN-γ to IL-4 production, whereas the CD4 T cells from wild-type peptide-primed mice produced IFN-γ. These data, taken together, suggest that the real variable in this system is the interaction of the peptide/MHC class II complex with the TCR, and that this can be adjusted either through altered binding to MHC, or through altered binding of the complex to a TCR.

Discussion

These studies show that the nature of the complex ligand-comprising peptide bound to an MHC class II mole-

Table 1. Summary of Characteristics of Murine Response to huColIV

| Type of response of CD4 T cells | MHC class II genotype | |
|---|-----------------------|----------|
| | <i>b</i> | <i>s</i> |
| Proliferation | - | +++ |
| T cell help for antibody formation (IgG1) | +++ | - |
| Cytokine expression | | |
| IL-2 | + | +++ |
| IFN-γ | - | +++ |
| IL-4 | ++ | - |
| IL-5 | ++ | - |

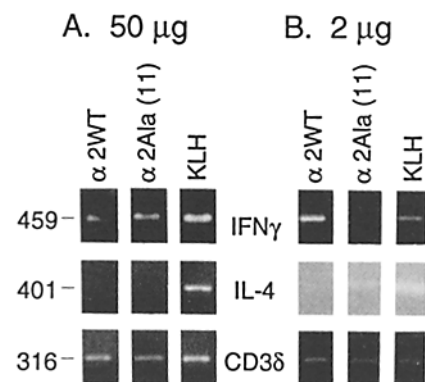


Figure 4. Increasing the priming dose changes the type of effector CD4 T cells generated in H-2^b mice. CD4 T cells from H-2^b (A.SW) mice primed with 50 μg (A) or 2 μg (B) of mutant or wild-type peptide were analyzed for cytokine mRNA expression, as described in Fig. 2. Supernatants were tested for IFN-γ production, as described in Materials and Methods, and yielded the following results: Priming with: 50 μg-wild-type peptide = 27 U/ml; α2Ala(11) mutant peptide = 6 U/ml; priming with: 2 μg-wild-type peptide = 18 U/ml; α2Ala(11) mutant peptide = < limits of detection. The limit of detection in this experiment was 0.5 U/ml rIFN-γ. No IL-4 was detected by bioassay, as has been reported previously (18).

cule and its interaction with the TCR can influence the differentiation of naive CD4 T cells into Th1-like or Th2-like cells. It appears that when used at a single dose, peptides that bind well to MHC class II molecules and/or to the TCR lead selectively to Th1-like cells, while peptides that bind less well prime Th2-like cells. It can be argued that CD4 T cells of different specificity are primed by wild-type and mutant peptides, both of which recognize the wild-type peptide upon secondary challenge. While this would account for the selective priming of Th1- and Th2-like cells, we feel that T cell specificity does not determine effector cell type, since mice carrying TCR transgenes can develop either Th1- or Th2-like responses (21, 22, 27).

The present studies show that similar effects occur with *in vivo* priming, which may account for the surprising finding that MHC class II genotype can control the functional differentiation of CD4 T cells in response to huColIV. The implication of this finding is twofold. Firstly, it suggests that signals delivered via the TCR can control the differentiation of CD4 T cells into their selective effector cell types. How these signals differ is not known; it may be a quantitative difference in the intensity of signaling, or it may reflect the types of altered signaling that account for differential responses of mature T cells to peptides and their variants (28–31). A simple view of the mechanism accounting for the generation of distinct effector functions is that the extent of TCR aggrega-

tion leads to different intracellular signals. This might be due to differences in the threshold of signals needed for distinct cytokine gene expression, which may in turn be controlled by TCR occupancy. Alternatively, it might be due to the differential participation of costimulatory molecules. If TCR aggregation is inefficient, there may be a corresponding deficiency in the induction of costimulatory molecules important in the selective generation of Th1 or Th2 T cells.

Secondly, these results suggest that high MHC class II/peptide density on the antigen-presenting cell surface favors Th1-like responses, while low ligand densities favor Th2-like responses. If this interpretation is correct, it has important implications for allergy. Allergens are not known to provoke inflammatory reactions that would generate the cytokines necessary to promote Th2-like differentiation; however, they are presented to the human immune system at very low dose, leading to IgE synthesis. Moreover, desensitization is generated by injecting successively higher doses of allergen. Likewise, the highest ligand densities are likely to be generated by B cells binding soluble protein antigens and by macrophages infected with intracellular pathogens. Priming in these cases appears to favor generation of Th1-like cells. Thus, the outcome of priming CD4 T cells can be determined, both by the cytokines present in the site of priming and by the antigen itself. Which of these is more important in a given immune response must be determined by direct analysis.

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References

- Kim, J., A. Woods, E. Becker-Dunn, and K. Bottomly. 1985. Distinct functional phenotypes of cloned Ia-restricted helper T cells. *J. Exp. Med.* 162:188–201.
- Mosmann, T.R., H. Cherwinski, M.W. Bond, M.A. Giedlin, and R.L. Coffman. 1986. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. *J. Immunol.* 136:2348–2357.
- Tite, J.P., M.B. Powell, and N.H. Ruddle. 1985. Protein-antigen specific I-A restricted cytolytic T cells: analysis of frequency, target cells susceptibility, and mechanism of cytolysis. *J. Immunol.* 135:25–33.
- Cher, D., and T. Mosmann. 1987. Two types of murine helper T cell clone. II. Delayed type hypersensitivity is mediated by Th1 clones. *J. Immunol.* 138:3688–3694.
- Stout, R.D., and K. Bottomly. 1989. Antigen-specific activation of effector macrophages by IFN γ producing (Th1) T cell clones. Failure of IL4 producing (Th2) T cell clones to activate effector function in macrophages. *J. Immunol.* 142:760–765.
- Killar, L., G. MacDonald, J. West, A. Woods, and K. Bottomly. 1987. Cloned Ia restricted T cells that do not produce IL4/BSF-1 fail to help antigen specific B cells. *J. Immunol.* 138:1674–1679.
- Stevens, T.L., A. Bossie, V.M. Sanders, R. Fernandez-Botran, R. Coffman, T.R. Mosmann, and E. Vitetta. 1988. Regulation of antibody isotypic secretion by subsets of antigen-specific helper T cells. *Nature (Lond.)* 334:255–258.
- Le Gros, G., S.Z. Ben-Sasson, R. Seder, F.D. Finkelman, and W.E. Paul. 1990. Generation of interleukin 4 (IL-4)-producing cells *in vivo* and *in vitro*: IL-2 and IL-4 are required for *in vitro* generation of IL-4-producing cells. *J. Exp. Med.* 172:921–929.
- Swain, S.L., A.D. Weinberg, M. English, and G. Huston. 1990. IL-4 directs the development of TH2 like helper effectors. *J. Immunol.* 145:3796–3806.
- Betz, M., and B.S. Fox. 1990. Regulation and development of cytochrome c-specific IL-4 producing T cells. *J. Immunol.* 145:1046–1052.
- Chatelain, R., K. Varkila, and R.L. Coffman. 1992. IL-4 induces a Th2 response in *Leishmania major*-infected mice. *J. Immunol.* 148:1182–1187.
- Nakajima, H., I. Iwamoto, S. Tomoe, R. Matsumara, H.

- Tomioka, K. Takatsu, and S. Yoshida. 1992. CD4⁺ T-lymphocytes and interleukin-5 mediate antigen-induced eosinophil infiltration into mouse trachea. *Am. Rev. Respir. Dis.* 146: 374-377.
13. Scott, P., E. Pearce, A. Cheever, R.L. Coffman, and A. Sher. 1989. Role of cytokines and CD4⁺ T cell subsets in the regulation of parasitic immunity and disease. *Immunol. Rev.* 112: 161-182.
 14. Gazzinelli, R.T., S. Hieny, T.A. Wynn, S. Wolf, and A. Sher. 1993. IL-12 is required for the T-lymphocyte-independent induction of interferon gamma by an intracellular parasite and induces resistance in T cell deficient hosts. *Proc. Natl. Acad. Sci. USA.* 90:6115-6119.
 15. Heinzl, F.P., D.S. Schoenhaut, R.M. Rerko, L.E. Rosser, and M.K. Gately. 1993. Recombinant interleukin 12 cures mice infected with *Leishmania major*. *J. Exp. Med.* 177:1505-1509.
 16. Sypek, J.P., C.L. Chung, S.E.H. Mayor, J.M. Subramanyam, S.J. Goldman, D.S. Sieburth, S.F. Wolf, and R.G. Schaub. 1993. Resolution of cutaneous leishmaniasis: interleukin 12 initiates a protective T helper type 1 immune response. *J. Exp. Med.* 177:1797-1802.
 17. Tite, J.P., H.G. Foellmer, J.P. Madri, and C.A. Janeway, Jr. 1987. Inverse Ir gene control of the antibody and T cell proliferative responses to human basement membrane collagen. *J. Immunol.* 139:2892-2898.
 18. Murray, J.S., J. Madri, J. Tite, S.R. Carding, and K. Bottomly. 1989. MHC control of CD4⁺ T cell subset activation. *J. Exp. Med.* 170:2135-2140.
 19. Murray, J.S., C. Pfeiffer, J. Madri, and K. Bottomly. 1992. MHC control of CD4 T cell subset activation. II. A single peptide induces either humoral or cell-mediated responses in mice of distinct MHC genotype. *Eur. J. Immunol.* 22:559-565.
 20. Pfeiffer, C., J. Murray, J. Madri, and K. Bottomly. 1991. Selective activation of Th1 and Th2 like cells in vivo. Response to human collagen IV. *Immunol. Rev.* 123:65-84.
 21. Seder, R.A., W.E. Paul, M.M. Davis, and B. Fazekas de St. Groth. 1992. The presence of interleukin 4 during in vitro priming determines the lymphokine-producing potential of CD4⁺ T cells from T cell receptor transgenic mice. *J. Exp. Med.* 176:1091-1098.
 22. Hsieh, C.S., A. Heimberger, J. Gold, A. O'Garra, and K. Murphy. 1992. Differential regulation of T helper phenotype development by IL-4 and IL-10 in α , β T-cell receptor transgenic system. *Proc. Natl. Acad. Sci. USA.* 89:6065-6069.
 23. Sette, A., J. Sidney, M. Albertson, C. Miles, S.M. Colon, T. Pedrazini, A.G. Lamont, and H.M. Grey. 1990. A novel approach to the generation of high affinity class II-binding peptides. *J. Immunol.* 145:1809-1813.
 24. Sette, A.S. Southwood, D. O'Sullivan, F.C. Gaeta, J. Sidney, and H.M. Grey. 1992. Effect of pH on MHC class II-peptide interactions. *J. Immunol.* 148:844-851.
 25. Fritz, R.B., M.J. Skeen, C.H. Chou, M. Garcia, and I.K. Egorov. 1985. Major histocompatibility complex-linked control of the murine immune response to myelin basic protein. *J. Immunol.* 134:2328-2332.
 26. Finnegan, A., M.A. Smith, J.A. Smith, J. Berzofsky, D.H. Sachs, and R.J. Hodes. 1986. The T cell repertoire for recognition of a phylogenetically distant protein antigen. Peptide specificity and MHC restriction of staphylococcal nuclease-specific T cell clones. *J. Exp. Med.* 164:897-910.
 27. Hsieh, C.S., S.E. Macatonia, C.S. Tripp, S.F. Wolf, A. O'Garra, and K.M. Murphy. 1993. Development of Th1 CD4⁺ T cells through IL-12 produced by Listeria-induced macrophages. *Science (Wash. DC).* 260:547-549.
 28. Evavold, B.D., and P.M. Allen. 1991. Separation of IL-4 production from Th cell proliferation by an altered T cell receptor ligand. *Science (Wash. DC).* 252:1308-1310.
 29. Rocken, M., K.M. Muller, J.H. Saurat, I. Muller, J.A. Louis, J.C. Cerottini, and C. Hauser. 1992. Central role for TCR/CD3 ligation in the differentiation of CD4⁺ T cells toward a Th1 or Th2 functional phenotype. *J. Immunol.* 148:47-54.
 30. Williams, M.E., A.H. Lichtman, and A.K. Abbas. 1990. Anti-CD3 antibody induces unresponsiveness to IL-2 in Th1 clones but not in Th2 clones. *J. Immunol.* 144:1208-1214.
 31. Racioppi, L., F. Ronchese, L.A. Matis, and R.N. Germain. 1993. Peptide-major histocompatibility complex class II complexes with mixed agonist/antagonist properties provide evidence for ligand-related differences in T cell receptor-dependent intracellular signaling. *J. Exp. Med.* 177:1047-1060.