Brief Definitive Report

MHC CONTROL OF CD4+ T CELL SUBSET ACTIVATION

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The specific immune response couples recognition of foreign antigens with a variety of effector mechanisms that are broadly classified as either humoral or cell mediated. Humoral immune responses are mediated by antibody produced by activated B cells while cell-mediated responses involve macrophage and cytolytic T cell activation. Both forms of immunity require participation of activated CD4+ T cells. Recent studies have shown that cloned lines of CD4+ T cells able to elicit effector responses characteristic of humoral or cell mediated immunity are of distinct subsets (1–3), producing different cytokines (4) that are believed to account for these activities. To examine whether the mechanism that determines the effector class of a given immune response involves normal correlates of cloned CD4+ subsets in vivo, we have studied an immune response in which the effector phenotype is controlled by a MHC class II gene. We show that different cytokines are produced by CD4+ T cells responding to the same antigen in mice of different MHC genotypes. Moreover, the CD4+ T cells responding in mice of different MHC genotypes also differ in their ability to induce antibody production. These data demonstrate that MHC class II genotype can control the functional outcome of an immune response.

Materials and Methods

Mice. A.SW/SnJ and A.BY/SnJ mice (female 6–8 wk old) were purchased from The Jackson Laboratory, Bar Harbor, ME.

Antigens. Type IV human collagen (collagen IV) was purified and characterized as previously described (5).

Lymph Node CD4+ T Cell Proliferation Assay. Lymph node cells were isolated from congenic mice immunized 10 d previously with 50 μg of collagen IV in CFA (H37Ra; Difco Laboratories, Detroit, MI) at the base of the tail (5). CD4+ T cells were prepared as described (6). CD4+ T cells were cultured for 96 h at 4×10^5 cells/well with 10^5 irradiated (3,000 rad) syngeneic T-depleted splenic APC/well as described previously (5).

Hapten-specific IgG Antibody Responses. 2×10^5 CD4+ T cells were mixed with 10^6 syngeneic T cell–depleted spleen cell from TNP-OVA–primed mice and varying concentrations of TNP-collagen IV (only the optimal concentration of 0.05 μg/ml is shown) in the presence or absence of 1 μg/ml purified (rat IgG1) mAb, 11B11, or mAb35 (isotype-matched control antibody). Serial dilutions of 6 d supernatants were incubated on TNP-BSA coated plates, followed by the addition of biotinylated antiisotype reagents, avidin-peroxidase, and finally

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substrate buffer. Monoclonal anti-TNP isotype controls were used to establish the specificity of the ELISA.

Analysis of Lymph Node CD4⁺ T Cell Blasts for IL4, IL2, and IFN-γ mRNA by In Situ Hybridization. Mice were immunized as above and lymph node cells cultured with 25 μg/ml collagen IV as described for Fig. 1. After 48 h cells were fractionated into CD4⁺ T cells as above and blast CD4⁺ T cells were obtained from the interface of 50–60% Percoll (Pharmacia Fine Chemicals, Uppsala, Sweden) by centrifugation. The procedure used for in situ hybridization has been described previously (6); >400 cells contained in >5 fields of view were counted for each slide under ×1,000 magnification. Background hybridization was determined with sense-strand probes.

Results and Discussion

Both humoral and cell-mediated protective immune responses are driven by activated CD4⁺ T cells. While cloned T cell lines differ in their functional capabilities, it is of interest to determine if similar functional specialization exists in normal CD4⁺ T cells. Previous studies of this question have examined susceptibility and resistance of mice to chronic infection with *Leishmania major* (7, 8); antibodies to cell surface molecules (9, 10); lectin binding (11); or cytokines produced by single CD4⁺ T cells (6). While these studies suggest that subsets of CD4⁺ T cells similar to those derived by cloned lines exist in vivo, no understanding of the mechanism of selecting distinct functional effects or subsets has been obtained. In this report, we examine the CD4⁺ T cell cytokine production and helper activity during a response to type IV human collagen (collagen IV), where it has been observed that mice expressing the MHC class II gene product I-A^s display strong lymph node antigen-specific T cell proliferation in the absence of significant antibody production, while all other genotypes tested lacked proliferative responses but produced abundant antigen-specific antibody (5). Previous studies of the immune response to collagen IV did not examine purified CD4⁺ T cells, leaving open the possibility that the differences observed reflected inhibition of responses by other cell types. We therefore examined the responses of purified CD4⁺ T cells from mice of different MHC genotypes for the ability to proliferate in response to collagen IV (Fig. 1) and to help B cells produce anti-TNP antibody in response to TNP-collagen-IV (Fig. 2 *a*). As shown, CD4⁺ T cells from mice expressing I-A^s (here, A.SW) proliferate in response to low doses of collagen IV, while CD4⁺ T cells from mice expressing I-A^b (here, the congenic strain A.BY) do not respond even to high doses of collagen IV. This difference is antigen specific, since CD4⁺ T cells from both

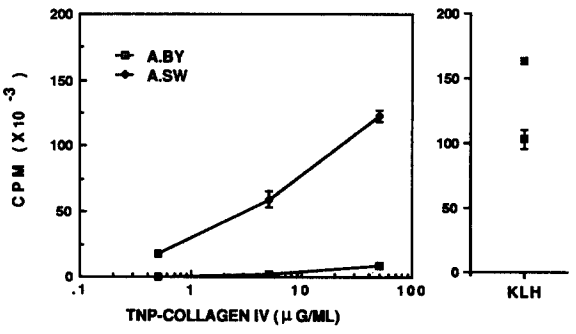

FIGURE 1. Lymph node CD4⁺ T cell proliferative response to human collagen IV. CD4⁺ T cells from I-A^s- and I-A^b-primed congenic strains were analyzed for recall proliferation to either collagen IV or 5 μg/ml KLH in vitro. Error bars represent the SD of triplicate wells; background proliferation (no antigen) was <5,000 cpm and is subtracted from these values. Representative data are shown. In separate experiments, I-A^s proliferative responses to collagen IV were consistently ≥ a log higher than I-A^b.

strains respond strongly to KLH (Fig. 1) and to PPD (5; data not shown). By contrast, $CD4^+$ T cells from $I\text{-}A^s$ mice do not help effectively for an IgG anti-TNP antibody response to TNP-collagen IV, while $CD4^+$ T cells from $I\text{-}A^b$ give very potent IgG anti-TNP antibody responses that are dominated by IgG1 antibodies (Fig. 2 *a*). This difference in the secretion of IgG1 anti-TNP antibody was observed at all TNP-collagen concentrations tested (data not shown). Since the secretion of IgG1 has been shown to be influenced by IL-4 (12, 13), it was important to determine if the IgG1 response to TNP-collagen IV required IL-4 production by T cells from $I\text{-}A^b$ mice. As is shown in Fig. 2 *b*, the mAb 11B11, which is specific for murine IL-4, significantly and specifically blocks the synthesis of IgG1 anti-TNP in this system. Thus, IL-4 production appears to be coupled to IgG1 antibody production in this system.

To further examine whether the dichotomy in responses to collagen IV seen between $I\text{-}A^s$ and $I\text{-}A^b$ mice reflected selective activation of Th-1-like or Th2-like $CD4^+$ T cells, respectively, $CD4^+$ T cells from immunized mice were restimulated with collagen IV in vitro, and the responding T cell blasts were assayed for IL-4, IL-2, and IFN-γ expression by in situ hybridization. These cytokines are selectively expressed by Th2 (IL-4 producing) and Th1 (IL-2/IFN-γ producing) subsets of $CD4^+$ T cell clones (4). It can be seen (Fig. 3) that the proliferative response to collagen IV in $I\text{-}A^s$ mice is accompanied by the expression of IFN-γ (Fig. 3 *b*) and IL-2 (Fig. 3 *a*) mRNA, but not expression of IL-4 (Fig. 3 *c*) mRNA (background hybridization was negligible; data not shown). By contrast, the response to collagen IV by T cells from $I\text{-}A^b$ mice is accompanied by the expression of IL-4 mRNA but not IFN-γ mRNA. These results have been confirmed by bioassays of supernatants from these and replicate cell cultures using cytokine-responsive T cell lines and specific mAbs to measure individual cytokines. We have consistently observed IFN-γ only in $I\text{-}A^s$

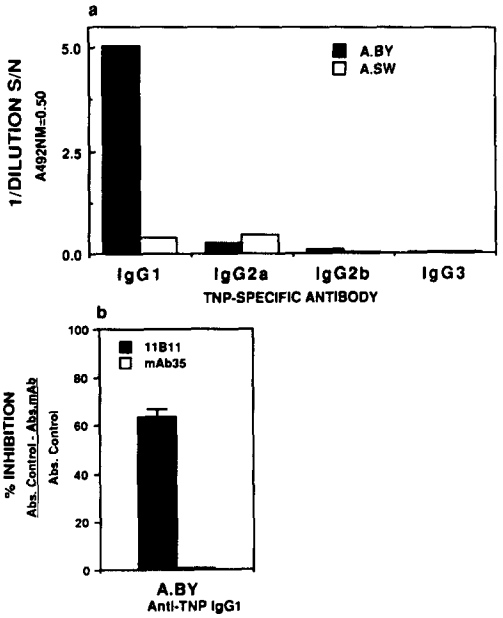

FIGURE 2. Secondary collagen IV-induced anti-TNP antibody responses in vitro. (*a*) In vitro TNP-specific IgG antibody responses. $CD4^+$ T cells from collagen-primed mice were analyzed for their ability to induce anti-TNP antibody secretion from TNP-primed B cells in the presence of TNP-collagen IV. (*b*) Anti-IL-4 inhibition of TNP-specific IgG1 antibody responses. Results are expressed as the reciprocal dilution giving an absorbance of 0.50 at 492 nm.

cell culture supernatants, IL-2 at low levels in I-A^b and high levels in I-A^s supernatants, and IL-4 when detected only in I-A^b culture supernatants (data not shown). Thus, the induced cytokine response of normal $CD4^+$ T cells to this antigen is determined by the MHC genotype of the mouse.

The present experiments confirm earlier studies in other experimental systems that functionally distinct sets of $CD4^+$ T cells arise selectively in an in vivo immune response (7, 8, 14). The selective activation of IL-4-producing cells in strains of mice that also produce antibody to collagen IV, and the fact that IL-4 production was not detected in strains of mice that do not produce specific antibody, support our previous observations with Th2 cloned lines (1) suggesting that IL-4 production correlates with helper function in specific antibody responses.

Whether a subdivision of $CD4^+$ T cells into Th1 and Th2 cells occurs before or as a result of antigenic stimulation is not yet clear. It has recently been suggested that IL-4-producing cells are a consequence of memory responses, i.e., secondary stimulation, whereas IL-2 producers dominate primary immune responses (9, 15, 16). The present results argue against a simple sequential mechanism, as we observed strong antigen-specific recall T cell proliferative responses that were dominated by IL-2/IFN-γ-producing $CD4^+$ T cells. Rather, the differential activation of antigen-specific Th1-like and Th2-like cells in mice of distinct class II MHC genotype strongly suggests that events involved in antigen presentation per se contribute to the selective activation of a particular subset of T cell.

There are three possible mechanisms by which class II MHC genotype might control the subset of $CD4^+$ T cells activated during a specific immune response,

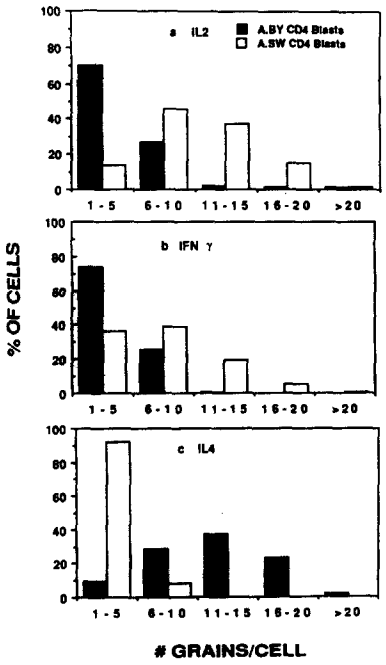

FIGURE 3. Lymphokine mRNA expression by collagen IV-primed $CD4^+$ T cells. Steady-state mRNA of IL-2 (*a*), IFN-γ (*b*), and IL-4 (*c*) was measured by in situ hybridization with ribo probes prepared from lymphokine-specific cDNA. Blast cells were obtained as described in Materials and Methods from collagen IV-primed I-A^s and I-A^b congenic mice and compared for lymphokine mRNA. The frequencies of positive cells recovered from the total lymph node cell populations were similar, i.e., 1.7% (A.BY) and 1.6% (A.SW), and indicate frequencies similar to those reported for antigen-stimulated IL-2- and IL-4-producing T cells as analyzed by limiting dilution analysis (16).

and each applies equally to any of several models of $CD4^+$ subset differentiation. If the effects observed reflect the binding of different peptides by different I-A alleles, then this implies that T cells of different functional capabilities also differ in their TCR repertoire. This conclusion is incompatible with optimal functioning of immune surveillance, since there is no a priori mechanism for determining if a particular epitope will be expressed on an intracellular or extracellular bacterium. However, in single cases like collagen IV, this mechanism could operate. Ligand density could also be controlled by MHC genotype, whether the same or different peptides are presented. It is possible the $CD4^+$ T cells of different functions are responsive to different densities of ligand, or that ligand density determines the functional maturation of a $CD4^+$ cell. Finally, all T cell activation appears to require both ligand/receptor interactions and accessory cell-derived second signals. IL-2-producing Th1 and IL-4-producing Th2 cells differ in this requirement, and evidence from in vivo studies are also consistent with this proposal. Class II MHC genotype could control the accessory cell used for priming. For instance, if a peptide binds poorly to class II MHC, then only cells internalizing large amounts of antigen could present this ligand; antigen-specific B cells, known to be required for priming proliferating but not helper T cells, are very efficient in this regard. By contrast, if a class II MHC product binds peptide well, then the small amounts of antigen apparently taken up by most macrophages could be effectively presented. Data from chimeric chickens (17) and transgenic mice (Flavell, R., personal communication) both suggest that priming of T cells able to provide specific help to B cells requires macrophage presentation of antigen/MHC complexes. Finally, Th1 clones are specifically inhibited by high concentrations of antigen (18), as would be achieved on B cells internalizing large amounts of a protein whose peptides bind well to class II MHC. Thus, achieving the correct ligand density on a suitable APC may be the critical determinant of effector cell activation, and this could be affected by MHC class II genotype.

In conclusion, our studies demonstrate that class II MHC genotype can control whether T cells responding to a given antigen make IL-2 and IFN-γ, or IL-4. There are several mechanisms that can explain this result. The critical issue now is to determine which of them is/are correct, and then to determine at what stage in $CD4^+$ T cell development this functional specialization occurs.

Summary

The present results demonstrate that $CD4^+$ T cells activated in the primary in vivo response to antigen produce distinct patterns of cytokines depending upon the MHC class II haplotype of the responding mice. $I\text{-}A^s$ mice were found to selectively activate IL-2/IFN-γ-producing $CD4^+$ T cells, whereas $I\text{-}A^b$ mice exhibited selective activation of IL-4-producing $CD4^+$ T cells in response to collagen IV. The effector response phenotype was found to correlate with the cytokine phenotype of $CD4^+$ T cells activated in vivo; IL-2/IFN-γ-producing cells giving rise to proliferative (cell-mediated) responses, IL-4-producing cells leading to secondary IgG (humoral) responses. Together the data support the notion that the outcome of a given immune response (e.g., protection vs. onset, tolerance vs. autoimmunity) may be determined in part by the type of $CD4^+$ T cells initially activated by antigen. Moreover, the present experiments demonstrate for the first time that polymorphism

in class II MHC can determine such selective activation of different cytokine-producing $CD4^+$ T cell phenotypes.

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