The Presence of Interleukin 4 during In Vitro Priming Determines the Lymphokine-producing Potential of CD4+ T Cells from T Cell Receptor Transgenic Mice

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Summary

To study the factors that determine whether CD4+ T cells produce interleukin 4 (IL-4) or interferon γ (IFN-γ) upon stimulation we used a system allowing naive T cells to be primed in vitro by specific antigen. Dense CD4+ T cells were purified from mice that expressed transgenes encoding a T cell receptor specific for pigeon cytochrome C peptide 88-104 in association with I-E^k. These T cells produced very limited amounts of IL-4 and IFN-γ upon immediate challenge with 88-104 and antigen-presenting cells (APC). However, after an initial "priming" culture in which they were incubated for 4 d in the presence of 88-104, APC, and 1,000 U/ml IL-4, the T cells acquired the capacity to produce substantial amounts of IL-4 upon rechallenge but made very little IFN-γ. Cells primed in the absence of IL-4 produced IFN-γ upon rechallenge but virtually no IL-4. The inhibitory effect of IL-4 on IFN-γ production did no appear to be mediated by the induction of IL-10 production since IL-10 addition to initial cultures did not suppress priming for IFN-γ production, nor did anti-IL-10 block the inhibitory effect of IL-4. IFN-γ itself did not increase priming for IFN-γ production, nor did anti-IFN-γ reduce such priming. IFN-γ did, however, diminish priming for IL-4 production when limiting amounts of IL-4 (100 U/ml) were used in the initial culture. The dominant effect of IL-4 in determining the lymphokine-producing phenotype of primed cells was observed with dendritic cells (DC), activated B cells, and I-E^k-transfected fibroblasts as APC. However, the different APC did vary in their potency, with DC being superior to activated B cells, which were superior to transfected fibroblasts.

Immune responses are often characterized by a polarization in the lymphokines that CD4+ T cells produce in vivo or in response to in vitro challenge (1–4), showing dominance of IL-4 or IFN-γ production, respectively. The choice of lymphokines produced in response to infectious agents can often determine whether that response will be protective or not. For example, T cells from mice that develop a progressive infection with *Leishmania major* mainly produce IL-4 upon antigenic challenge, while mice in whom lesions heal mount an immune response characterized by the production of IFN-γ (1, 2). Thus, understanding the factors that determine which lymphokines will be produced is of considerable importance. Recent work has demonstrated that the presence of IL-4 during stimulation of resting T cells with polyclonal activators can give rise to T cell populations capable of high IL-4 production on secondary challenge (5–7). Furthermore, treating mice with anti-IL-4 at the time of infection with *L. major* causes mice that normally develop a response dominated by IL-4 to produce IFN-γ instead and to heal their leishmanial lesions (8, 9). These results strongly suggest that lymphokines themselves may play a central role in regulating the spectrum of lymphokines produced in an immune response.

To examine the priming of T cells during activation by specific antigen rather than polyclonal activators, we used small dense T cells from mice transgenic for TCR α and β chain genes that encode a TCR specific for peptide 88-104 of pigeon cytochrome C in the context of I-E^k class II MHC molecules. These T cells respond to stimulation with APC and peptide 88-104 by secreting IL-2 but little IL-4 or IFN-γ. We show here that IL-4 is essential for in vitro antigen priming for IL-4 production upon restimulation and that such IL-4 treatment profoundly inhibits the development of antigen-

specific IFN-γ producers. This essential role for IL-4 was observed using dendritic cells (DC),[1] activated B cells, and I-E^k-transfected fibroblasts as APC.

Materials and Methods

Animals. TCR transgenic mice were produced by microinjection of genomic TCR constructs into [B10.S × C57BL/6J]F_1 fertilized eggs using standard techniques (10). The rearranged α and β chain gene constructs were made from DNA derived from the 5C.C7 T cell clone (11), and were cointegrated and expressed under the control of the endogenous 3′ β chain enhancer (Fazekas de St. Groth et al., manuscript in preparation). The -I line used in all the experiments detailed below was established and maintained by backcrossing to B10.A mice originally derived from The Jackson Laboratory (Bar Harbor, ME). All the mice used in experiments were homozygous for H-2^a and heterozygous for the integration of TCR α and β chains. APC were prepared from virus-free B10.A female mice, 8–12 wk old, obtained from the Division of Cancer Treatment, National Cancer Institute (Frederick, MD).

Tissue Culture Medium. RPMI 1640 supplemented with 10% heat-inactivated FCS, penicillin (100 U/ml), streptomycin (100 μg/ml), 1 mM sodium pyruvate, L-glutamine (2 mM), and 2-ME (50 μM) was used for all stimulations and assays.

Peptide. A peptide with the sequence corresponding to residues 88–104 of pigeon cytochrome C (KAERADLIAYLKQATAK) was synthesized by the National Institutes of Allergy and Infectious Diseases (NIAID) Biological Resources Branch (Bethesda, MD).

Recombinant Lymphokines. Human IL-2 was a gift of Cetus Corp. (Emeryville, CA). IL-2 activity was defined in terms of "Cetus Units." One Cetus unit is equivalent to ~0.3 ng. Mouse rIL-4 was was obtained by a baculovirus expression system, utilizing a vector into which the IL-4 gene had been inserted by C. Watson (Laboratory of Immunology, NIAID). One unit of IL-4 is equivalent to ~0.5 pg. Mouse rIL-10 was a generous gift of DNAX (Palo Alto, CA). Mouse IFN-γ was purchased from Genzyme (Cambridge, MA).

Preparation of Accessory Cells. Highly enriched DC were prepared from spleen cells of B10.A mice by allowing 2 × 10^8 cells in 10 ml complete RPMI to adhere to plates (3025; Falcon Labware, Oxnard, CA) for 2 h at 37°C. Nonadherent cells were then discarded, plates washed three times with complete RPMI, and 10 ml of fresh medium was added. After overnight incubation at 37°C, the DC became nonadherent and were collected, centrifuged over a 50% Percoll (Pharmacia Fine Chemicals, Piscataway, NJ) solution, and assessed for purity by flow cytometric analysis. They were found to be 75–85% enriched for DC as judged by staining with biotinylated mAb 33D1 and streptavidin-PE. Cells were briefly incubated with the anti-FcγRII/III antibody 2.4G2 (12) before adding 33D1 to block binding of 33D1 to such Fc receptors.

Activated B cells were prepared from spleens of B10.A mice as follows: single cell suspensions, depleted of erythrocytes by ACK lysing buffer, were incubated with a mixture of anti-Thy-1.2 (HO-13.4) (13), anti-CD4 (GK1.5) (14), and anti-Lyt-2 (3.155) (15) hybridoma supernatants for 15 min on ice, and then for 45 min at 37°C in the presence of mouse rat anti-Ig antibody (MAR 18.5, ATCC T1B216) and rabbit complement (Cedarlane, Westbury, NY). Cells were further enriched by harvesting the 60/70% fraction from a discontinuous Percoll density gradient. B cells were activated for 48 h with 5 μg/ml of affinity-purified goat anti-mouse IgM (Southern Biotechnology, Birmingham, AL) and 100 U/ml of murine rIL-4. Activated B cells were also prepared by stimulating resting B cells for 48 h with LPS (20 μg/ml). The purity of B cells was assessed by flow cytometry analysis using FITC-conjugated anti-B220 (6B2) (16) antibody. Generally, these cells were >99% B220 positive.

Fibroblasts transfected with I-E^k were a generous gift of Dr. Ronald Germain (NIAID).

Preparation of T Cells. Lymph node $CD4^+$ T cells from transgenic mice were prepared as previously described (5). Briefly, lymph node cells were incubated with antibodies to CD8 (2.43) (15), B220 (6B2), and/or I-A^k (11-3.25) (17), and cells expressing these markers were removed by magnetic bead depletion. The residual cells were centrifuged on a discontinuous Percoll density gradient. The 60/70% fraction was harvested and subsequently used for all primary cultures. The purity and transgene expression of the $CD4^+$ T cells were verified by flow cytometric analysis using two-color immunofluorescence with PE-labeled anti-CD4 and FITC anti-Vα11 (RR8-1) obtained from Pharmingen. In most experiments, >90% of CD4 T cells expressed Vα11.

Primary and Secondary Stimulation of Transgenic T Cells. Primary stimulation was carried out by adding 5–6 × 10^5 small $CD4^+$ T cells to individual wells of 24-well plates in a total volume of 1 ml with accessory cells, peptide, and lymphokine as described in Results. After 36–48 h, cells were transferred to 5-ml dishes and supplemented with fresh medium for an additional 48 h to allow for further expansion. T cells were then washed three times and 10^5 cells restimulated with peptide and APC in a total volume of 200 μl in microtiter plates. 36 h later, supernatants were collected and assayed for lymphokine.

Measurement of Lymphokine Production. IFN-γ was assayed by specific two-site ELISAs (18, 19), with reference standard curves using known amounts of rIFN-γ. Lymphokine-dependent cell lines (CT.4S), an IL-4-dependent line (20), and CTLL, an IL-2-dependent cell line (21), were used to measure IL-4 and IL-2 production, respectively, using serial dilutions of supernatants and comparing responses to those elicited by known amounts of murine rIL-4 and human IL-2 as standards. Anti-IL-4 antibody (11B11) was added to the CTLL cells to block any effect of IL-4 on these cells. Lymphokine production is reported in terms of the total lymphokine-producing capacity of cells derived from a given priming culture (U/culture); the amount of lymphokine produced by 10^5 cells in the "secondary" culture is multiplied by the number of cells harvested from the priming culture divided by 10^5.

Antibodies. Purified monoclonal rat anti-mouse IL-4 (11B11) (22) was prepared by Verax Corporation (Lebanon, NH). Rat anti-mouse IFN-γ (XMG 1.2) (23), PgP-1 (1m7) (24), and LECAM-1 (MEL14) (25) were purchased from Pharmingen. Monoclonal rat anti-mouse IL-1 receptor (type 1) was purchased from Genzyme. 33D1 (26) was obtained from American Type Culture Collection (Rockville, MD). Purified rat anti-mouse IL-10 (SXC 1) was a generous gift of Dr. Maureen Howard (DNAX Institute of Molecular and Cellular Biology, Palo Alto, CA) (27).

Results

Characterization and Responses of Initial Cell Populations. Lines of mice carrying α and β TCR genomic constructs derived from the helper T cell line 5C.C7 were derived by standard methods (Fazekas de St. Groth, B., et al., manuscript in preparation). More than 90% of $CD4^+$ lymph node T

[1] *Abbreviation used in this paper:* DC, dendritic cells.

cells derived from these mice expressed the TCR transgenes, as indicated by staining with mAbs to Vα11 and Vβ3. Dense CD4+ T cells were purified. More than 90% of these cells were Mel 14+ (LECAM-1), CD44low (Pgp-1), indicating that the cell population was highly enriched in naive T cells (data not shown).

Freshly isolated transgenic CD4+ T cells produced small amounts of IL-4 or IFN-γ (<50 U/ml/10^5 cells) upon immediate challenge with 1 μM of the relevant peptide pigeon cytochrome C (88-104) and 5 × 10^3 DC, as illustrated in Fig. 1. Results were generally similar with a wide range of peptide concentrations and with the use of activated B cells as APC. These cells could be primed under conditions described below to produce much larger amounts of either lymphokine (>4,000 U/ml/10^5 cells) upon rechallenge with the same concentration of peptide and the same number of DC. The data on cells primed in vitro are shown here only to illustrate the poor production of these lymphokines by naive cells.

In other experiments, the frequency of freshly prepared transgenic T cells that could be acutely stimulated to produce IL-4 (28) was shown to be <1/2,000 (data not shown), reinforcing the conclusion that among unprimed T cells from transgenic donors, IL-4 is produced by very few cells.

IL-4 Present during Priming Determines the Pattern of Lymphokines Produced upon Subsequent Challenge. CD4+ T cells (10^6/ml) from transgenic donors were primed in vitro by culture with DC (2 × 10^4/ml), peptide (10 μM), and IL-2 (10 U/ml) for 4 d, with or without IL-4 (1,000 U/ml) or anti-IL-4 (10 μg/ml). Generally, priming cultures were established with a total of 5 × 10^5 cells. If either peptide or APC were omitted from the priming culture, viable T cell yield was <10^5 at 4 d, whereas in the presence of APC and peptide, >10^7 cells were obtained. Among cells obtained after priming with peptide and APC, >92% were Mel-14^{lo}, and >99% CD44^{hi}, indicating that the great majority of cells had been activated as a result of the priming process (data not shown). This was true whether IL-4, anti-IL-4, or neither had been added to the priming culture.

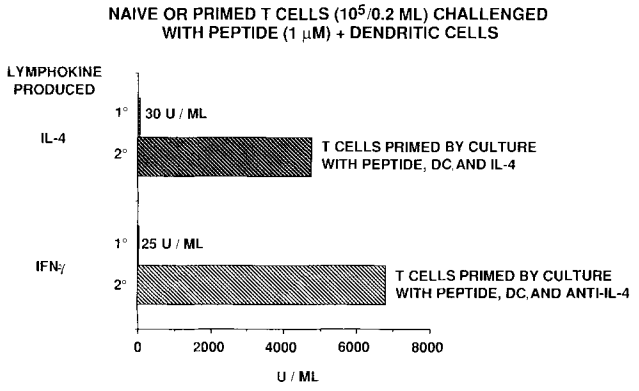

Figure 1. Transgenic T cells produce little IL-4 and IFN-γ upon primary challenge. CD4+ transgenic T cells were prepared from naive mice and stimulated with dendritic cells and peptide for 36 h. Culture supernatants were harvested and tested for production of IL-4 and IFN-γ (primary response [1°]). Naive CD4+ T cells were also cultured for 4 d with dendritic cells, peptide, and either IL-4 or anti-IL-4. Cells were washed, and restimulated with dendritic cells and peptide. 36 h later, supernatants were harvested and tested for IL-4 and IFN-γ production (secondary response [2°]). To prime for IL-4 production, IL-4 (1,000 U/ml) was added to the initial culture, while priming for IFN-γ production involved the addition of anti-IL-4 (10 μg/ml) to the initial culture.

The primed cells were washed and restimulated in 96-well plates at 10^5/0.2 ml with fresh DC (2 × 10^4/well) and peptide (1 μM). Supernatants were collected 36 h later and concentrations of IL-4 and IFN-γ were measured (Fig. 2). The amount of IL-4 and IFN-γ produced in the secondary culture was multiplied by the yield of cells from primary culture to determine lymphokine production per priming culture. Large amounts of IL-4 were produced by cells that had been primed in the presence of IL-4; no other population produced significant amounts of IL-4. By contrast, IFN-γ was produced in substantial amounts by cell populations that had been primed without the addition of IL-4 or to which anti-IL-4 had been added during priming; cells primed in the presence of IL-4 produced <100 U IFN-γ/culture upon rechallenge (Fig. 2).

Similar results were obtained when primed T cells were restimulated with activated B cells and peptide, or with immobilized anti-CD3, indicating that the pattern of lymphokine production was determined by the priming conditions rather than by the restimulation conditions. Furthermore, the pattern of production of IL-2 in secondary cultures was similar to that of IFN-γ. Cells primed with IL-4 produced little or no IL-2 upon secondary challenge, whereas cells primed in the presence of anti-IL-4 produced substantial amounts of IL-2 (data not shown). We also tested the priming of transgenic T cells by intact cytochrome C, which gave results essentially the same as those obtained with peptide.

IL-10 Does Not Inhibit Priming for IFN-γ Production. IL-10 has been demonstrated to diminish IL-2 and IFN-γ production by T cell clones of the T_{H1} type, and is believed to exert its effects by acting on macrophages and possibly other APC (29). To determine whether IL-10 would inhibit priming of transgenic T cells for IFN-γ production and whether the effect of exogenous IL-4 inhibiting such priming might require endogenous production of IL-10, priming was carried out with concentrations of IL-4 ranging from 0 to 1,000 U/ml in the presence of either IL-10 or anti-IL-10 antibody (Fig. 3). In the absence of IL-4, IL-10 did not significantly diminish priming for IFN-γ production, nor did it cause priming for IL-4 production. Anti-IL-10 caused a modest enhancement in priming for IFN-γ production in the experiment illustrated in Fig. 4, but this effect was not observed in other experiments, suggesting that endogenously produced IL-10 was not suppressing priming for IFN-γ production. At the optimal concentration of IL-4 (1,000 U/ml), priming for IL-4 was not affected by the presence of IL-10 or anti-IL-10. No priming for IFN-γ was observed under these conditions even in the presence of anti-IL-10, indicating that the effect of IL-4 in preventing priming for IFN-γ production is independent of IL-10.

At an intermediate concentration of IL-4 (100 U/ml),

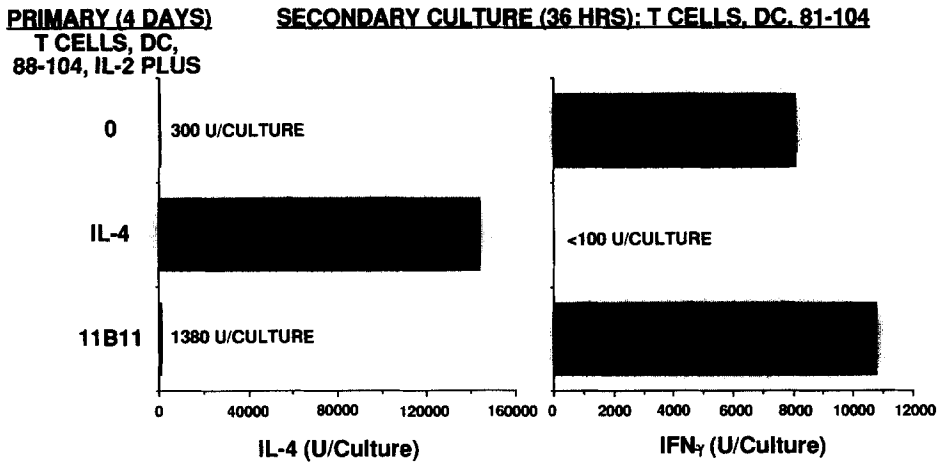

Figure 2. IL-4 is required for priming for IL-4 production and inhibits priming for IFN-γ production. Naive CD4+ transgenic T cells (10^6) were primed by stimulation for 4 d with dendritic cells (2×10^4), peptide (10 μM), and IL-2 (10 U/ml), with or without IL-4 (1,000 U/ml) or anti-IL-4 (10 μg/ml). Cells were washed and recultured at 10^5/200 μl with dendritic cells (2×10^4) and peptide (1 μM) for 36 h. Supernatants were harvested and tested for production of IL-4 and IFN-γ. Results are reported as lymphokine production per priming culture (cell yield/10^5 multiplied by lymphokine production per 10^5 cells).

priming for IL-4 production was diminished and inhibition of priming for IFN-γ production was only partial. Once again, IL-10 did not significantly enhance the inhibition of priming for IFN-γ production, nor did anti-IL-10 relieve the inhibition.

Effect of IFN-γ on Priming Transgenic T Cells. IFN-γ has been reported to inhibit the appearance of T cell clones capable of producing IL-4, suggesting that it might have an effect on the priming process (30). To examine this, transgenic T cells were primed in the presence of of 0, 100, or 1,000 U/ml of IL-4 together with 0, 100, 500, and 2,500 U/ml of IFN-γ (Fig. 4). IFN-γ did not diminish priming for IL-4 production when an optimal concentration of IL-4 (1,000 U/ml) was used, nor did anti-IFN-γ enhance priming for IL-4 production in the absence of IL-4. However, when a suboptimal priming concentration of IL-4 (100 U/ml) was used, IFN-γ caused a substantial reduction in priming at all doses from 100 to 2,500 U/ml, indicating that under certain conditions IFN-γ as well as IL-4 can regulate priming for IL-4 production.

We also wished to determine whether IFN-γ played a role in the priming for IFN-γ production. In the absence of IL-4, priming for IFN-γ production was not significantly enhanced by the addition of IFN-γ, nor did anti-IFN-γ inhibit such priming. Furthermore, IFN-γ did not overcome the inhibitory effects of IL-4 in preventing priming for IFN-γ production. These results indicate that although IFN-γ plays an inhibitory role in priming for IL-4 production when suboptimal concentrations of IL-4 are used, it does not have any striking effect on priming for IFN-γ production.

The Effect of IL-4 in Determining Lymphokine-producing Capacity Is Independent of APC Type. To determine whether the regulatory role of IL-4 in determining lymphokine-producing phenotype only held when certain types of APC were used for priming, we used DC, B cells activated by culture with LPS, and a fibroblast line that had been transfected with the I-E^k gene as APC in primary cultures (Fig. 5). In each case, the presence of IL-4 during the primary response prepared the cells to produce IL-4 upon rechallenge with DC and peptide. In these experiments, cells cultured with anti-IL-4 during the primary response produced IFN-γ but little IL-4 upon rechallenge. In the experiment illustrated in Fig. 5, the ratio of APC to T cells was 1:50 for DC, 1:50 for activated B cells, and 1:5 for fibroblasts, so that the superiority of DC over B cells and of B cells over fibroblasts in degree of priming suggests that the type of APC affects the magnitude of priming rather than the pattern of lymphokines that are produced. In other experiments, a variety of densities of APC have been utilized with generally similar results. Moreover, B cells activated with anti-IgM plus IL-4, T cell–depleted spleen cell populations, and nonadherent spleen cell populations used as APC gave similar results. These ex-

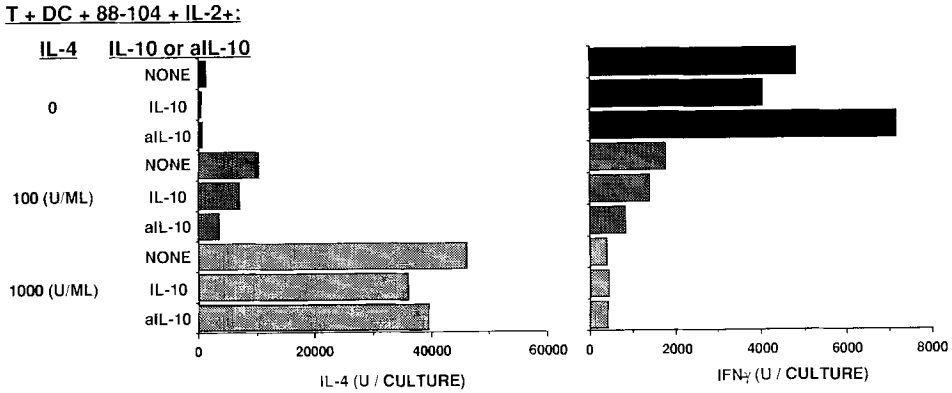

Figure 3. IL-10 does not inhibit priming for IFN-γ production. CD4+ transgenic T cells (5×10^5) were cultured with dendritic cells (2.5×10^4), 88-104 (0.1 μM), and IL-2 (10 U/ml) in the presence of 0–1,000 U/ml IL-4 and IL-10 (100 U/ml) or anti-IL10 (10 μg/ml). Supernatants were assessed for lymphokine production after restimulation, and results are reported as described in the legend to Fig. 2.

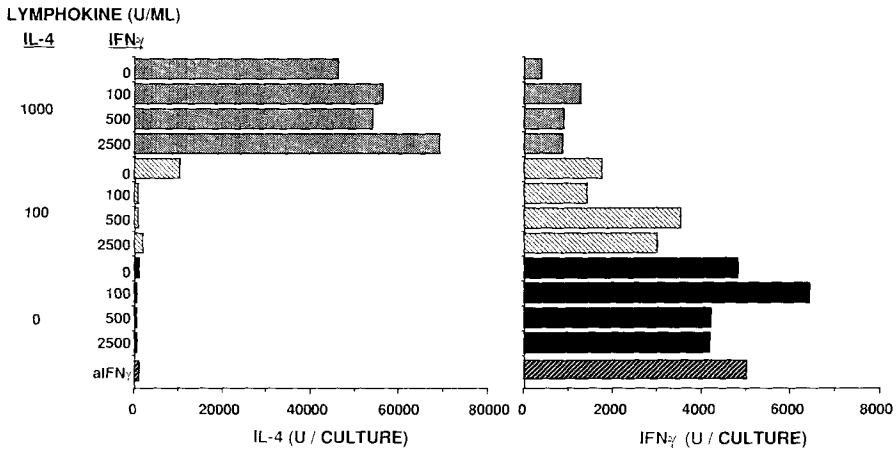

Figure 4. Relative effects of IL-4 and IFN-γ on priming for lymphokine production. CD4+ T cells from transgenic mice were cultured as described in the legend to Fig. 4 in the presence of 0–1,000 U/ml IL-4 and 0–2,500 U/ml IFN-γ. IL-4 and IFN-γ were measured in supernatants obtained after restimulation, and results are reported as described in the legend to Fig. 2. Anti-IFN-γ (10 μg/ml) was also added to cells primed without IL-4 or IFN-γ.

periments indicate that the presence or absence of IL-4 during the priming process determines the pattern of subsequent lymphokine production, at least for the range of APC tested.

Discussion

There is now considerable evidence that few resting T cells from naive donors produce IL-4 in response to initial stimulation, and that production of IFN-γ is also meager in such cell populations (5, 27, 31–34). Rather, the production of these lymphokines depends upon a priming event. Previous studies with polyclonal activators demonstrated a critical role for IL-4 itself in determining the capacity of primed cells to produce IL-4 (5–7). The current studies make use of a model in which antigen specific T cells are primed by recognition of their cognate ligands on APC and thus allows a more physiologic approach to the determination of lymphokine-producing phenotype than is possible with polyclonal stimuli.

There are several possible mechanisms acting independently or together that may commit a naive T cell population to produce particular lymphokines upon subsequent stimulation: (*a*) Lymphokines themselves may regulate the ability of naive T cells to become committed to production of various lymphokines, as already demonstrated by in vitro priming with polyclonal stimuli (5–7, 35). (*b*) Different types of APC may express distinct "costimulatory" molecules or express different amounts of class II MHC molecules that may affect the priming for distinct lymphokines. (*c*) The degree of stimulation through the TCR may cause the cell to differentiate down one or another pathway (36, 37). (*d*) It is possible that there is a direct link between the specificity of the TCR and the type of lymphokine that the cell may be primed to produce. This could be achieved through thymic selection. (*e*) Factors contributed by the antigen (or by the "agent" bearing the antigen) may have a critical deterministic role, presumably by acting on one or another of the cells that participate in the interaction. Such action may be indirect in that it recruits one of the other mechanisms discussed above.

Several of these possibilities can be directly addressed using an in vitro priming system in which the T cells are derived from TCR transgenic mice. Using this model, we show that the bulk of dense CD4+ T cells from naive transgenic donors behave as if they are resting cells. Most particularly, upon immediate challenge with peptide and various types of APC, they produce small amounts of IL-4 or of IFN-γ, but, as we describe in some detail, appropriate priming causes the appearance of a vastly greater capacity to produce one or another of these lymphokines.

The most striking result is the dominant effect of IL-4 in determining the pattern of lymphokines produced upon sub-

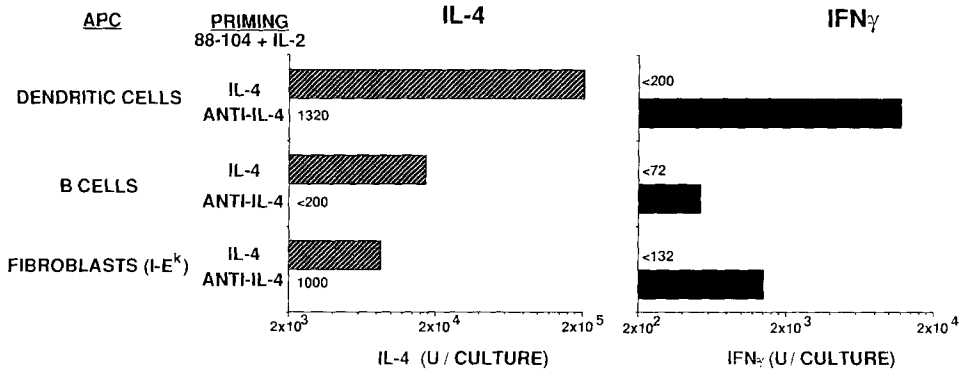

Figure 5. Lymphokine and APC control of priming for IL-4 and IFN-γ production. CD4+ T cells from transgenic donors were primed for 4 d under the same conditions as those described in the legend to Fig. 3, except that activity of dendritic cells (2×10^4) as APC was compared with that of LPS-activated B cells (2×10^4) or I-E^k-transfected L929 cells (fibroblasts) (2×10^5). 36 h later supernatants were harvested and assessed for production of IL-4 and IFN-γ. Results are reported as described in the legend to Fig. 2.

sequent in vitro stimulation. When IL-4 is present during priming at a concentration of 1,000 U/ml, restimulation of these cells results in production of large amounts of IL-4 and of little or no IFN-γ or IL-2. The concentration of IL-4 used (1,000 U/ml = $\sim 0.4 \times 10^{-10}$ M) would appear to be potentially within a physiologic range, since the IL-4 receptor binds IL-4 with an equilibrium constant of $\sim 10^{10}$ M^{-1} (38). Indeed, even lower concentrations of IL-4 (100 U/ml; $\sim 0.04 \times 10^{-10}$ M) were capable of inducing priming for IL-4 production, although they only partially inhibited the development of IFN-γ-producing capacity.

IL-10 has been reported to be an inhibitor of the production of IL-2 and IFN-γ (39), raising the possibility that IL-10 might mediate the effect of IL-4 in inhibiting priming for IFN-γ and IL-2 production. This possibility was ruled out by demonstrating that addition of IL-10 in the place of IL-4 in the priming cultures did not lead to inhibition of IFN-γ-producing activity and that anti-IL-10 antibody did not block the effect of IL-4 in inhibiting priming for IFN-γ (Fig. 3). These results thus point to the direct action of IL-4 as an inhibitor of priming for IFN-γ production.

In contrast to a requirement for IL-4 to allow subsequent IL-4 production, we failed to identify a lymphokine necessary for subsequent IFN-γ production. The addition of IFN-γ neither enhanced priming for IFN-γ-producing capacity, nor did anti-IFN-γ diminish this priming. In other studies, we failed to observe an effect of exogenous TGF-β on priming for IFN-γ production, although we have not fully excluded a role for endogenously produced TGF-β (R. A. Seder, et al., unpublished observations). Nonetheless, these initial results suggest the possibility that the acquisition of the capacity to produce IFN-γ as a result of priming may represent the "default" pathway of differentiation. The addition of IL-4 appears to block this differentiation and to commit the cells to develop into IL-4 producers.

Our studies on the importance of IL-4 for the acquisition of the capacity to subsequently produce IL-4 are consistent with in vivo studies by Sadick et al. (8) and more recently Chatelain et al. (9), in which the injection of anti-IL-4 mAbs at the time of *L. major* infection allows susceptible mice to develop a protective response marked by IFN-γ production. Furthermore, injections of IL-4 were reported to cause mice that are normally resistant to *L. major* to become susceptible and to develop T cells that produce IL-4 in response to antigen stimulation (9). It has also been shown that injection of anti-IL-4 at the time of immunization with hemocyanin results in a reduction in the frequency of IL-4-producing antigen-specific T cells and concurrently increases IFN-γ production (S. Z. Ben-Sasson et al., manuscript submitted for publication). These results provide very strong evidence of the physiological role of IL-4 in determining the pattern of lymphokine production.

If IL-4 is an important physiologic agent in controlling priming for IL-4 and IFN-γ production, it is logical to ask what is its source in vivo. Indeed, primary cultures do produce some IL-4 by 36 h of culture, as shown in Fig. 1, and capacity is increased by day 3 of initial culture. Although the amounts are insufficient for optimal priming for IL-4 production in vitro, it is possible that conditions in vivo may be such that the concentration achieved locally is adequate to initiate the positive feedback loop required for substantial IL-4 production in subsequent responses. Among T cells, two major sources for such initial IL-4 production can be considered. T cells that had been previously primed, possibly by cross-reactive antigens, could produce IL-4 upon antigenic challenge. Additionally, Bendelac et al. (40) have shown that $CD4^+$, $CD44^{bright}$ thymocytes are highly enriched in cells that produce IL-4 in response to immobilized anti-CD3, and that they retain this capacity to produce IL-4 for several days after emigrating to the periphery. These cells could respond to antigenic challenge with a burst of IL-4 production and thus provide the stimulus required for the longer lived T cells to develop into IL-4 producers.

In addition, we have recently shown that $Fc\epsilon RI^+$ cells in spleen and bone marrow are highly enriched in cells of the basophil and/or mast cell lineage that are potent producers of IL-4 when stimulated by crosslinkage of FcεRI or FcγRIII (41–43). It is possible that under conditions in which these cells are activated or increased in number, for example in helminthic infections, they provide a source of sufficient IL-4 to amplify IL-4 production by T cells.

There has been extensive work using different types of APC to stimulate proliferation and lymphokine production from established $CD4^+$ T cell clones. Gajewski et al. (44) showed that B lymphocytes are effective in stimulating T_{H2}-type clones to proliferate, while splenic-adherent cells were effective APC for clones of the T_{H1}-type (44). Chang et al. (45) reported that B cells used as APC cause T_{H2} clones to proliferate suboptimally unless IL-1 was added, whereas proliferative responses to macrophages as APC did not require exogenous IL-1. However, lymphokine production was similar using either cell type as APC and did not require IL-1 (44). Our results indicate that among the APC we have tested (DC, activated B cells, $I\text{-}E^k$-transfected fibroblasts, and T-depleted spleen [data not shown]) all stimulated qualitatively similar lymphokine responses in that IL-4 was required for priming for IL-4 production and suppressed priming for IFN-γ production, while priming for IFN-γ production occurred without the need to add an exogenous cytokine. However, in quantitative terms, DC were superior to activated B cells, which were superior to $I\text{-}E^k$-transfected fibroblasts.

As discussed above, there is evidence that IL-1 is a necessary costimulator for proliferation of some T_{H2} clones, although it is not required for lymphokine production. Koide and Steinman (46) showed that dendritic cells produce virtually no IL-1 and questioned the need for IL-1 for lymphocyte activation. To address the question of whether IL-1 is required either for priming T cells to produce IL-4 or for expansion of cells that become IL-4 producers, we performed experiments in which exogenous IL-1 or anti-IL-1 receptor antibody were added to the priming cultures. Adding exogenous IL-1 in the absence of IL-4 did not prime cells to become IL-4 producers, and adding an antibody to the type 1 IL-1 receptor in the presence of IL-4 did not inhibit priming for IL-4 produc-

tion (data not shown). Moreover, it has been shown that addition of the IL-1 receptor antagonist to naive T cells in the presence of a polyclonal stimulator and IL-4 does not effect the development of IL-4-producing cells (A. K. Abbas, personal communication). Thus, IL-1 does not appear to play a major role in the generation of lymphokine-producing cells from naive T cells.

Our results also show that cells producing either IL-4 or IFN-γ can have identical T cell receptors. Several TCR α and β chains of T cell clones specific for antigens of *L. major* have been sequenced and instances have been observed in which identical sequences for both chains occurred in clones that produced IL-4 or IFN-γ (S. L. Reiner, personal communication), further arguing against a strong effect of receptor structure on lymphokine producing capacity.

From these studies we conclude that IL-4 is a critical regulator of the commitment of $CD4^+$ T cells to the production of IL-4 and to the inhibition of their production of IFN-γ and IL-2. This conclusion naturally leads to the question of what regulates the production of IL-4 involved in this initial decision process and how different forms of immunization establish different patterns of lymphokine-producing T cells.

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