*Proc. Natl. Acad. Sci. USA*
Vol. 89, pp. 6065–6069, July 1992
Immunology

# Differential regulation of T helper phenotype development by interleukins 4 and 10 in an αβ T-cell-receptor transgenic system

CHYI-SONG HSIEH*, AMY B. HEIMBERGER*, JENNIFER S. GOLD*, ANNE O'GARRA†, AND KENNETH M. MURPHY*‡

*Department of Pathology, Washington University School of Medicine, St. Louis, MO 63110; and †DNAX Research Institute of Molecular and Cellular Biology, Inc., Palo Alto, CA 94304

*Communicated by Emil R. Unanue, February 24, 1992*

ABSTRACT **To address the mechanisms controlling T helper ($T_h$) phenotype development, we used DO10, a transgenic mouse line that expresses the αβ T-cell receptor from an ovalbumin-reactive T hybridoma, as a source of naive T cells that can be stimulated *in vitro* with ovalbumin peptide presented by defined antigen-presenting cells (APCs). We have examined the role of cytokines and APCs in the regulation of $T_h$ phenotype development. Interleukin 4 (IL-4) directs development toward the $T_{h2}$ phenotype, stimulating IL-4 and silencing IL-2 and interferon γ production in developing T cells. Splenic APCs direct development toward the $T_{h1}$ phenotype when endogenous IL-10 is neutralized with anti-IL-10 antibody. The splenic APCs mediating these effects are probably macrophages or dendritic cells and not B cells, since IL-10 is incapable of affecting $T_h$ phenotype development when the B-cell hybridoma TA3 is used as the APC. These results suggest that early regulation of IL-4 and IL-10 in a developing immune response and the identity of the initiating APCs are critical in determining the $T_h$ phenotype of the developing T cells.**

The recognition of distinct patterns of cytokine production among murine T-cell clones has led to the concept that the heterogeneity of $CD4^+$ T cells (1–3) underlies the development of divergent immune responses to antigens (4, 5). Type 1 T helper ($T_{h1}$) clones, producing interleukin 2 (IL-2) and interferon γ (IFN-γ), elicit delayed-type hypersensitivity (DTH) responses and activate macrophages against intracellular pathogens (6, 7). $T_{h2}$ cells, by secreting IL-4, IL-5, and IL-6, provide more efficient help for B-cell activation, antibody production, and switching to the IgE and IgG1 isotypes (8–11). The potential for T cells with these distinct phenotypes to develop *in vivo*, particularly in settings of chronic infections, such as *Leishmania* (4, 12), *Nippostrongylus brasiliensis* (5), or *Schistosoma mansoni* (13), may explain how DTH and humoral responses to antigen can be mutually exclusive.

IL-4 has been implicated as necessary for production of the IL-4-producing, $T_{h2}$ phenotype *in vitro* (14–16). Swain *et al.* (17) have identified transforming growth factor β as influencing *in vitro* development toward $T_{h1}$ cells. Gajewski and Fitch (18) have proposed that IFN-γ skews toward $T_{h1}$ development, based on the antiproliferative effects of IFN-γ on $T_{h2}$ clones (18), and on the proportions of $T_{h1}$ versus $T_{h2}$ clones isolated in the presence of IFN-γ (19). *In vivo*, IL-4 and IFN-γ have been similarly shown to regulate $T_h$ responses to *Leishmania* infection (12, 20). Other cytokines, such as the $T_{h2}$-derived cytokine IL-10, which inhibits cytokine synthesis by $T_{h1}$ clones (21, 22), have not been examined for effects of $T_h$ phenotype development.

The transgenic mouse line DO10 (23) expresses the αβ T-cell receptor (TCR) from the T-cell hybridoma DO11.10 (24), reactive to the chicken ovalbumin (Ova)-(323–339) peptide. The majority of $CD4^+$ T cells from this mouse line express the antigen-specific TCR clonotype from DO11.10 and can be identified with the anti-clonotypic monoclonal antibody (mAb) KJ1-26 (25). This report examines the effects of the cytokines IL-4 and IL-10 and antigen-presenting cells (APCs) in the developmental regulation of $T_h$ phenotype. We show that naive T cells expressing a single αβ TCR clonotype can differentiate to both $T_{h1}$ and $T_{h2}$ phenotypes under certain cytokine and APC conditions when stimulated by fixed levels of peptide antigen *in vitro*. Therefore, neither antigenic specificity nor antigen density exclusively determine phenotype. Our results show that the cytokines IL-4 and IL-10 as well as the initiating APCs can influence phenotype emergence.

## MATERIALS AND METHODS

**T-Cell Purification and Culture.** Splenocytes from unimmunized transgenic mice (23) were isolated on a Ficoll gradient (Histopaque-1119, Sigma). Cells not adherent to nylon wool were subjected to complement lysis using CA4 (pan anti-class II major histocompatibility complex proteins) (A. L. Glasebrook, Eli Lilly) and 3.155 (anti-CD8) (26), followed by a 2-hr adherence at 37°C. The remaining cells were >95% pure $CD4^+$ T cells as shown by flow cytometry and gave no proliferative response or cytokine production (<0.2 unit of IL-2 per ml) to Ova-(323–339) without added APCs.

T cells ($2.5 \times 10^5$ per well) were stimulated in 24-well plates with 0.3 μM Ova-(323–339) presented by various irradiated APCs: BALB/c splenocytes [2600 rads (1 rad = 0.01 Gy), $5 \times 10^6$ per well], SCID (severe combined immunodeficiency) splenocytes (2600 rads, $2.5 \times 10^6$ per well), or the H-$2^{d/k}$-expressing B-cell hybridoma TA3 (10,000 rads, $5 \times 10^5$ per well) (27). Effects of cytokine or antibody additions were determined by assaying cytokine production by T cells restimulated without added cytokine or antibody.

**Cytokines and Anti-Cytokine mAbs.** IL-10 (28) was used at 10 units/ml. Anti-IL-10 mAb SXC-1 (29) and anti-IL-4 mAb 11B11 (30) were used at 10 μg/ml. Recombinant murine IL-4 (Genzyme) was used at 200 units/ml or as indicated.

**Cytokine Assays.** IFN-γ, IL-4, IL-2, and IL-5 were quantitated with a sandwich ELISA (31). For IL-4, the anti-IL-4 mAb 11B11 (10 μg/ml) was used as the primary (coat) antibody, with polyclonal rabbit anti-IL-4 (1:20,000) (Genzyme) as the secondary antibody. IL-2 was assayed similarly with a rat anti-mouse IL-2 mAb (1.25 μg/ml) (Genzyme) and polyclonal rabbit anti-IL-2 (1:5000) (Genzyme). IL-5 was assayed with anti-IL-5 mAb TRFK-5 as the primary antibody

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: APC, antigen-presenting cell; Ova, ovalbumin; mAb, monoclonal antibody; $T_h$, T helper; TCR, T-cell receptor; IL, interleukin; IFN, interferon.

‡To whom reprint requests should be addressed.

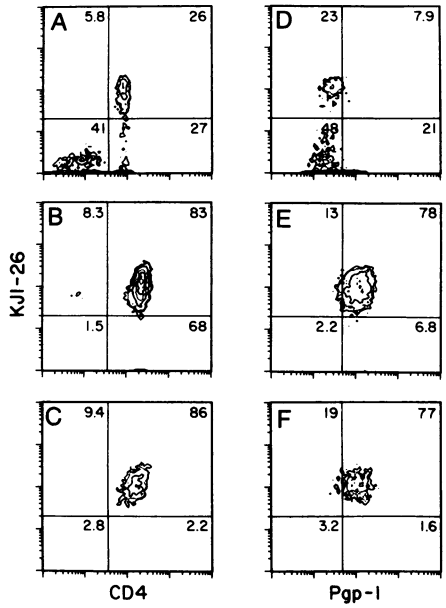


FIG. 1. Expression of identical TCR clonotype by DO10 transgenic T cells and $T_{h1}$ and $T_{h2}$ cell lines. Unpurified transgenic splenocytes (*A* and *D*), a $T_{h1}$ cell line (*B* and *E*), and a cloned $T_{h2}$ line (*C* and *F*) were stained for surface expression of DO11.10 clonotypic TCR with the mAb KJ1-26 and for CD4 (*A–C*) or Pgp-1 (*D–F*) and were analyzed by two-color flow cytometry. $T_{h1}$ cell lines were generated by extended culture (more than three stimulations) in the presence of anti-IL-10 mAb SXC-1. Cloned $T_{h2}$ cells were derived from cultures initiated in the presence of IL-4. Numbers indicate the percentage of cells in each quadrant (e.g., in *A*, 26% of the cells show surface expression of DO11.10 TCR and CD4, and 41% express neither).

and biotinylated mAb TRFK-4 (32) as the secondary antibody. Staining reagents, biotinylated goat anti-rabbit (1:20,000) and avidin D–peroxidase conjugate (1:5000) (Vector Laboratories), were developed with 3,3',5,5'-tetramethylbenzidine (Medic Biotech, Foster City, CA).

**Flow Cytometry.** Two-color flow cytometric analysis used biotinylated KJ1-26 and streptavidin–phycoerythrin conjugate (Southern Biotechnology Associates, Birmingham, AL) and fluorescein-conjugated GK1.5 (anti-mouse-CD4; Becton Dickinson) or IM7 (anti-mouse Pgp-1; Pharmingen, San Diego).

## RESULTS

**T Cells from Nonimmunized TCR-Transgenic Mice Are Naive.** Pgp-1, a putative memory-cell marker (33), was not expressed by freshly isolated T cells from nonimmunized transgenic mice but was expressed at high levels after *in vitro* stimulation with Ova and acquisition of either the $T_{h1}$ or the $T_{h2}$ phenotype (Fig. 1). Freshly isolated transgenic T cells did not express the high-affinity IL-2 receptor, also consistent with a naive surface phenotype (data not shown) (34). Further, T cells from nonimmunized mice stimulated *in vitro* with Ova-(323–339) presented by BALB/c splenocytes produced primarily IL-2, with low to undetectable IL-4 and IFN-γ production, characteristic of naive T-cell populations (Fig. 2*A*; Table 1) (34, 35). When these T cells were subsequently reactivated, they acquired the capacity to produce large amounts of IL-4 or IFN-γ (Fig. 2 *B* and *C*).

Table 1. Effects of cytokines and anti-cytokine-mAbs on the emergence of $T_h$ phenotype experiment of Fig. 2

| | IL-2, units/ml | | | IL-5, pg/ml | | |
|---|---|---|---|---|---|---|
| Addition | 1 | 2 | 3 | 1 | 2 | 3 |
| IL-4 | 130 | 27 | 4 | <1.0 | 9.9 | 140.2 |
| 11B11 | 187 | 101 | 88 | 1.6 | <1.0 | 2.0 |
| IL-10 | 110 | 84 | 109 | <1.0 | 2.0 | 25.7 |
| SXC-1 | 124 | 101 | 112 | <1.0 | 1.7 | 6.8 |
| None | 202 | 109 | 122 | <1.0 | 2.4 | 20.6 |

IL-2 and IL-5 production is shown for stimulations 1–3.

**Regulation of $T_h$ Phenotype by IL-4.** IL-4 has been reported to favor the development of a $T_{h2}$ phenotype in other systems (14, 15). Addition of IL-4 (200 units/ml) to a primary T-cell activation mixture rapidly led to a strong $T_{h2}$ phenotype, with high IL-4 and IL-5 secretion and markedly reduced IFN-γ and IL-2 secretion compared with controls (Fig. 2 *B* and *C*; Tables 1 and 2). Neutralizing IL-4 with mAb 11B11 (10 μg/ml) during primary T-cell activation resulted in the $T_{h1}$ phenotype with strong IFN-γ and IL-2 production and low to undetectable IL-4 and IL-5 production (Fig. 2 *B* and *C*; Tables 1 and 2). The $T_{h1}$ phenotype was also obtained when the anti-IL-4 receptor mAb M1 was used to block IL-4 receptor activation during primary T-cell activation (data not shown) (36).

The ability of anti-IL-4 mAb to produce the $T_{h1}$ phenotype suggested the presence of endogenous IL-4 in primary T-cell cultures activated with antigen. We therefore measured the IL-4 produced by activation of primary T cells with antigen and BALB/c splenocytes (Fig. 3). While high levels of IL-2 were produced by day 2 (Fig. 3*A*), low to undetectable levels of IL-4 were present on day 2 after primary stimulation (Fig. 3*C*). By days 3 and 4, IL-4 had accumulated to 5–15 units/ml. This endogenous level of IL-4 produced T-cell lines with an intermediate cytokine secretion pattern, without strong skewing toward either phenotype (Table 2). Addition of as little as 25 units of IL-4 per ml produced significant skewing toward the $T_{h2}$ phenotype, with 50–200 units/ml giving

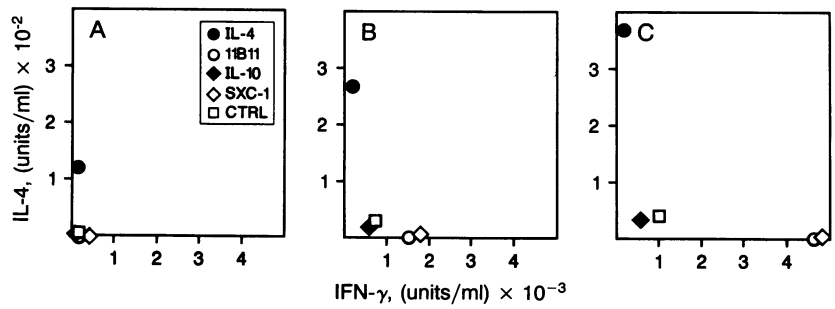


FIG. 2. Effects of cytokines and anti-cytokine mAbs on the emergence of $T_h$ phenotype. Shown are cytokine profiles measured by ELISA on day 2 of the primary (*A*), secondary (*B*), or tertiary (*C*) stimulation. The primary stimulation mixture contained added cytokine. Naive T cells were purified and stimulated with Ova-(323–339) peptide presented by BALB/c splenocytes. Cultures were maintained with either IL-4, the anti-IL-4 mAb 11B11, IL-10, the anti-IL-10 mAb SXC-1, or no cytokines (control, CTRL). To determine the cytokine profile, T cells previously maintained with added cytokine were washed and restimulated without added cytokine (*B* and *C*). This experiment was repeated four times, with similar results.

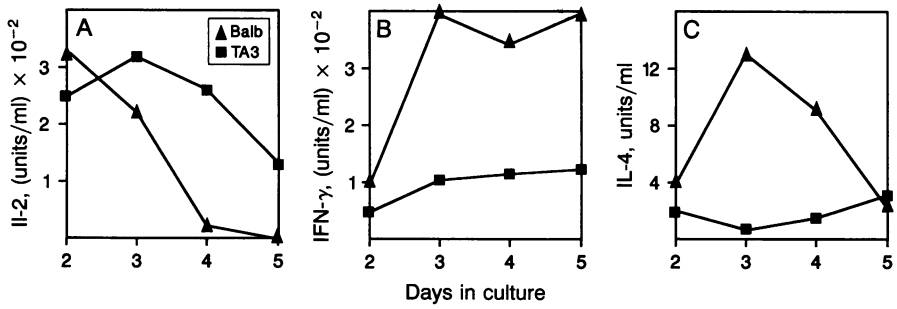


FIG. 3. Cytokine levels during the primary activation of naive T cells. Naive T cells were purified and activated with Ova peptide presented by BALB/c splenocytes or TA3 cells. Supernatant was harvested from identical wells on the day indicated and assayed by ELISA.

maximal effects (Table 2). IL-4 accumulation was lower in primary culture initiated by TA3 rather than BALB/c splenocytes as APCs (Fig. 3).

**IL-4 Does Not Influence the Phenotype of Committed T Cells.** Given the profound effects of IL-4 or 11B11 on phenotype during the primary stimulation (Fig. 2*B*), we determined whether these effects would operate on more-differentiated T-cell lines. IL-4 was added to either the first, second, or third stimulation of cultures previously maintained with 11B11. Cytokine production by IL-4-induced and noninduced control T-cell lines was determined, and the results are expressed as a ratio of cytokine production by IL-4-induced versus noninduced lines (Table 3). The strong enhancement of IL-4 production induced by IL-4 was most effective during the primary stimulation. This suggests that $T_{h1}$ precursors quickly lose their ability to respond to a signal from IL-4.

**IL-10 Exerts Distinct Regulatory Influences on Phenotype Development.** IL-10 was originally described as a $T_{h2}$-derived factor that inhibited cytokine synthesis by $T_{h1}$ but not $T_{h2}$ clones (22). Addition of IL-10 (10 units/ml) during T-cell activation did not upregulate IL-4 or IL-5 production, or inhibit IL-2 production, in contrast to the effect of IL-4 (Fig. 2; Table 1). Higher doses of IL-10 decreased IFN-γ production slightly but had no effect on IL-4 production (data not shown). Neutralization of endogenous IL-10 with the mAb SXC-1 (10 μg/ml) during T-cell activation markedly increased IFN-γ production and reduced IL-4 and IL-5 production (Fig. 2; Table 1). While neutralization of either IL-4 or IL-10 markedly enhanced IFN-γ production, only the addition of IL-4, and not IL-10, increased IL-4 and IL-5 production. This difference suggests that IL-4 and IL-10 may act through different cellular mechanisms. We therefore determined the abilities of different APCs to mediate the effects of IL-4 and/or IL-10 on T-cell phenotype development.

APCs that were able to initiate and maintain the proliferation and differentiation of transgenic T cells included I-$A^d$-transfected fibroblasts (data not shown), the B-cell hybridoma TA3, BALB/c splenocytes, and SCID splenocytes, which lack B and T cells (Fig. 4). Purified resting B cells, activated peritoneal macrophages (37), and the macrophage-like cell lines J774, P388D1, and WEHI-3 were relatively poor APCs for naive T cells and were unable to propagate T-cell cultures (data not shown).

The effect of IL-4 addition on phenotype development was independent of the APCs used to initiate and maintain T cells. Thus, when BALB/c splenocytes, SCID splenocytes, and TA3 B-hybridoma cells were used, addition of IL-4 induced a strong $T_{h2}$ phenotype, with high levels of IL-4 and little or no IFN-γ production (Fig. 4 *A*, *C*, and *E*). Thus, in the presence of IL-4, B cells may influence a response toward the $T_{h2}$ phenotype but are not essential for development of this phenotype. For each APC tested, neutralization of IL-4 in the primary activation led to T-cell lines that were later unable to produce IL-4 but did secrete IFN-γ. Even the B hybridoma TA3 allowed development toward the $T_{h1}$ phenotype when IL-4 was neutralized, although the production of IFN-γ was markedly reduced compared with splenic APCs (Fig. 4*C*).

In contrast to the APC-independent effects of IL-4, the effects of IL-10 depended on the APCs used to initiate T-cell activation. When irradiated BALB/c or SCID splenocytes were used as APCs, neutralization of IL-10 with SXC-1 markedly enhanced IFN-γ production and inhibited IL-4 production (Fig. 4 *B* and *F*). However, when the B-cell hybridoma TA3 was used to activate and propagate primary T cells, neutralization of IL-10 had little effect on the developing cytokine profile, producing a $T_{h2}$ phenotype similar to that of the TA3 control culture (Fig. 4*D*).

The effects of IL-4 on $T_h$ phenotype development are dominant over those of IL-10. Thus, addition of IL-4 to T cells activated by antigen and BALB/c splenocytes generated the $T_{h2}$ phenotype both when IL-10 was added to the system and when endogenous IL-10 was neutralized by the addition of SXC-1 (Fig. 5*A*). Neutralization of endogenous IL-4 by the addition of 11B11 led to the $T_{h1}$ phenotype, although the level of IFN-γ production continued to be regulated by IL-10 (Fig. 5*A*). This implies that IL-10 is not required for development of the $T_{h2}$ phenotype, although it

Table 2. Titration of IL-4 effect on the development of $T_h$ cells toward distinct phenotypes

| Addition | Conc., units/ml | IL-2, units/ml | IFN-γ, units/ml | IL-4, units/ml |
|---|---|---|---|---|
| 11B11 | — | 310 | 5000 | 0 |
| None | — | 238 | 1873 | 329 |
| IL-4 | 12.5 | 164 | 603 | 891 |
| | 25.0 | 107 | 631 | 1236 |
| | 50.0 | 20 | 326 | 1983 |
| | 100.0 | 21 | 369 | 2129 |
| | 200.0 | 23 | 358 | 1838 |

Purified naive transgenic T cells were stimulated twice with Ova-(323–339) peptide presented by BALB/c splenocytes in the presence of 11B11 (10 μg/ml), IL-4 (as indicated), or no cytokine. The cells were restimulated without addition of IL-4 or 11B11, and supernatant was harvested on day 2 for cytokine analysis. This experiment was done twice with similar results.

Table 3. Capacity of IL-4 to induce a $T_{h2}$ phenotype is diminished after the primary stimulation

| Stimulation with IL-4 | Ratio of cytokine synthesis (IL-4 vs. control cultures) | | |
|---|---|---|---|
| | IL-2 | IFN-γ | IL-4 |
| 1 | 0.79 (85) | 0.17 (2099) | 114.6 (<5) |
| 2 | 0.66 (285) | 0.33 (7500) | 9.4 (<5) |
| 3 | 1.06 (122) | 0.77 (6621) | 4.0 (<5) |

IL-4 (200 units/ml) was added to T-cell cultures during the first, second, or third *in vitro* stimulation. Prior to IL-4 addition, cultures were stimulated in the presence of 11B11. Results are expressed as ratio of cytokine production after restimulation by T cells to which IL-4 was added compared to control cultures stimulated in the presence of 11B11 during the first or the first and second stimulations. Values >1 indicate an induction of cytokine production caused by IL-4. Absolute cytokine production by control cultures (units/ml) is shown in parentheses and shows a progression to a $T_{h1}$ phenotype. Data are representative of three experiments.

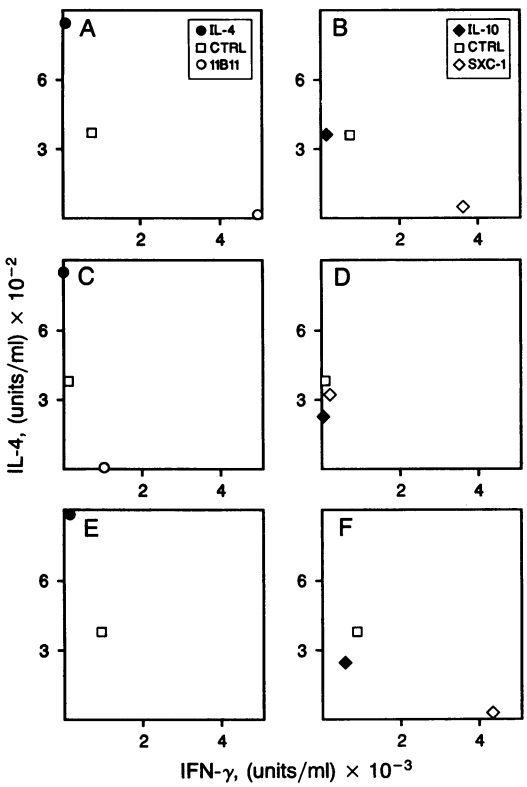


FIG. 4. Distinct regulatory influences on phenotype development by IL-4 and IL-10. Shown are cytokine profiles from the third stimulation of T cells influenced by both cytokines and APCs. Naive T cells were purified and stimulated with Ova peptide. BALB/c splenocytes (*A* and *B*), the B hybridoma TA3 (*C* and *D*), and SCID splenocytes (*E* and *F*) were used as APCs. T cells were maintained in the presence of added IL-4, 11B11, IL-10, SXC-1, or no cytokines (control, CTRL). IL-4 and 11B11 (*A*, *C*, and *E*) were not added during this stimulation. IL-10 and SXC-1 (*B*, *D*, and *F*) were added in this stimulation, but we have found in subsequent experiments that the continued presence of IL-10 or SXC-1 has little effect by the third stimulation. The combination of SCID APCs and 11B11 was not done in this experiment. Other experiments showed that the effects of 11B11 are comparable when either SCID or BALB/c splenocytes are the APCs. These conditions were examined at least three times, with similar results.

may inhibit $T_{h1}$ development. When TA3 cells were used as APCs, IL-10 did not affect IFN-γ production during phenotype emergence, and IL-4 continued to dominate phenotype development (Fig. 5*B*). Thus, neutralization of IL-4 produced a $T_{h1}$ phenotype with TA3 cells as APCs, with or without manipulation of IL-10 levels. Again, these $T_{h1}$ cells produced lower IFN-γ levels than when BALB/c splenocytes were used as APCs (Fig. 5*B*). These findings underscore a fundamental difference in mechanism of action between IL-4 and IL-10 for T-cell phenotype development.

## DISCUSSION

This transgenic system provides for physiologic activation of T cells by antigen–major histocompatibility protein complexes presented on APCs. A further advantage is that the T-cell population appears uniformly naive and has a single TCR with homogeneous affinity for Ova antigen. Other systems have employed *in vivo* primed (16), allogeneic (14), or mitogen-activated (14, 15) T cells, which may not be homogeneous and may include memory or differentiated T cells. We have shown that naive T cells with identical TCRs can develop into both $T_{h1}$ and $T_{h2}$ phenotypes under certain controlled cytokine and APC conditions. Initially, we exam-

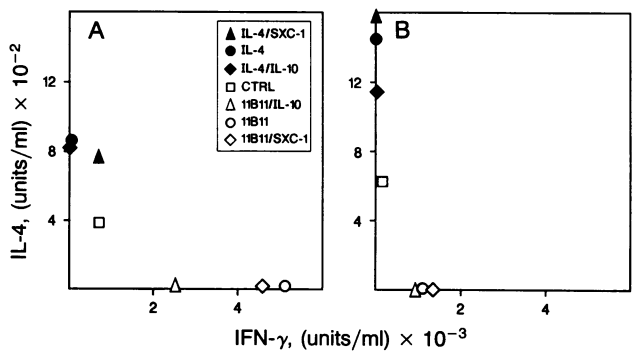


FIG. 5. IL-4 dominates IL-10 in influencing phenotype development. BALB/c splenocytes (*A*) or TA3 B-hybridoma cells (*B*) were used as APCs to prime purified naive T cells with antigen. Levels of IL-4 and IL-10 were regulated through combinations of cytokine and anti-cytokine mAbs. IL-4 or 11B11 was added alone or with IL-10 or SXC-1 during the previous stimulations. The cells were washed and restimulated without added cytokine. A culture without added cytokine (CTRL) is included for reference. This experiment was done twice, with similar results.

ined several cytokines for effects on phenotype development. We found unremarkable effects for additions of IL-1, anti-IL-1 antibodies, tumor necrosis factor α, and IFN-γ (data not shown). Neutralization of IFN-γ during *in vitro* priming skewed the response toward the $T_{h2}$ phenotype (data not shown). In this report, we have focused on the role of IL-4 and IL-10 and the role of APCs in this developmental system. The data show that IL-4 acts directly to promote $T_{h2}$ development, whereas IL-10 acts to inhibit the capacity of some APCs to drive $T_{h1}$ development.

Evidence from both *in vivo* and *in vitro* models suggests that cytokines, such as IL-4, IFN-γ, and transforming growth factor β, have a major role in $T_h$ development (17–19, 38–40). IL-4 enhances IL-4 production in heterogeneous T cells activated by concanavalin A or anti-CD3 (14, 15). Our studies add to the understanding of the effects of IL-4 on phenotype development. First, these data show that the effects of IL-4 occur regardless of the APCs used to activate naive T cells. Thus, IL-4 addition leads to the $T_{h2}$ phenotype when antigen is presented by BALB/c splenocytes, SCID splenocytes, or TA3 B-hybridoma cells. Second, these effects of IL-4 are most marked in the primary activation of uncommitted T cells and are not seen in stimulation of T cells previously activated in the absence of IL-4 (Table 3). The effects of IL-4 on development in this system occur at relevant concentrations (i.e., in the range produced by T cells in a primary stimulation; Table 2) and can be blocked by anti-IL-4-receptor antibodies.

Studies of fully differentiated cloned T-cell populations led some to suggest that APCs may determine the phenotype of responding T cells (38–40). Gajewski *et al.* (40) propose that B cells and macrophages, which provided optimal proliferative responses for cloned $T_{h2}$ and $T_{h1}$ cells, respectively, might direct the development of these phenotypes. Others argue that macrophages promote $T_{h2}$ development due to production of IL-1, a costimulator for $T_{h2}$ cells (39, 41), whereas B cells promote development of neither $T_h$ phenotype. All of these arguments strictly apply only to the proliferative requirements of fully differentiated $T_h$ clones and may not be germane to the developmental requirements of naive T cells. We have demonstrated that at least under certain cytokine conditions, the APC does not exclusively restrict development toward either phenotype. *In vivo*, the cytokine production from many cell types may contribute to phenotype development. Thus, $T_{h1}$ as well as $T_{h2}$ cells can develop when the initiating APC population is either B-cell-deficient (SCID splenocytes) or entirely B-cell-like (TA3) (Fig. 4). This suggests that B cells or macrophages may help

direct the phenotype of an immune response but are not exclusively required for development of either phenotype. Thus, development can be directed by extremes of IL-4 during initiation by several types of APCs. For example, IL-4 production from nonspecific immune cells, such as mast cells or basophils, could potentially bias the direction of $T_h$ phenotype development (42).

In this system, IL-4 was produced endogenously at moderate levels during primary T-cell activation (Fig. 3). Without manipulation, these levels of IL-4 do not strongly skew development toward either the $T_{h1}$ or the $T_{h2}$ phenotype. Under these conditions, certain APCs preferentially direct development toward one phenotype. Further, for some APCs, this capacity is modulated by IL-10. For example, the ability of splenic APCs, probably macrophages or dendritic cells, to strongly direct development toward the $T_{h1}$ phenotype is unmasked by neutralizing endogenously produced IL-10 (Fig. 4 *B* and *F*). Thus IL-10, a cytokine produced by $T_{h2}$ cells, macrophages, and Ly-1$^+$ B cells (43, 44), may suppress the development of $T_{h1}$-type responses. In contrast, without cytokine manipulations, the B-cell hybridoma TA3 preferentially induces a $T_{h2}$-like phenotype and does not induce a $T_{h1}$ phenotype even when IL-10 is neutralized (Fig. 4 *C* and *D*). These phenomena could be attributed to factors such as B7 (45, 46), expressed by some but not all APCs. IL-10 may operate by regulating the expression of such factors on APCs.

In *Leishmania major* infections, $T_{h1}$ responses correlate with resolution of disease, whereas $T_{h2}$ responses result in exacerbation of disease. Administration of an anti-IL-4 antibody during *Leishmania* infection of BALB/c mice confers a healer, $T_{h1}$-like phenotype (12), while administration of anti-IL-10 antibody does not (47), consistent with our demonstration *in vitro* that IL-4 can dominate IL-10 in affecting phenotype development. Administration of anti-IFN-$\gamma$ antibodies produces a nonhealer phenotype in normally resistant (healer) strains (12), consistent with our preliminary *in vitro* finding that neutralization of IFN-$\gamma$ leads to a $T_{h2}$ response. *In vivo* administration of IFN-$\gamma$ (12) does not permanently alter the $T_h$ response toward *Leishmania*, consistent with our finding that *in vitro* addition of IFN-$\gamma$ (1000 units/ml) does not alter $T_h$ phenotype significantly from controls (data not shown). However, while we find that additions of IL-4 consistently lead to a strong $T_{h2}$ phenotype, administration of IL-4 *in vivo* fails to convert a resistant strain to a nonhealer phenotype (47). Our *in vitro* system, therefore, can reflect *in vivo* $T_h$ development and is a useful model for examination of the individual cellular interactions and cytokine regulation of $T_h$ development.

We thank D. Y. Loh (St. Louis) for generous support and advice, R. D. Schreiber (St. Louis) for ELISA reagents and anti-cytokine antibodies, K. Moore (Palo Alto, CA) for recombinant IL-10 and mAb SXC-1, M. P. Beckman (Seattle) for M1 anti-IL-4 receptor antibody, and E. R. Unanue, T. L. Murphy, and C. T. Weaver (St. Louis) for critical reading of the manuscript. This work was supported by National Institutes of Health Grants 1 PO1 AI31238-01 and 5 T32 GM07200-17, a career development award from the International Juvenile Diabetes Foundation, and a grant from the Lucille P. Markey Foundation.

1. Mosmann, T. R., Cherwinski, H. M., Bond, M. W., Giedlin, M. A. & Coffman, R. L. (1986) *J. Immunol.* **136,** 2348–2357.
2. Cherwinski, H. M., Schumacher, J. H., Brown, K. D. & Mosmann, T. R. (1987) *J. Exp. Med.* **166,** 1229–1244.
3. Bottomly, K. (1988) *Immunol. Today* **9,** 268–274.
4. Scott, P., Natovitz, P., Coffman, R. L., Pearce, E. & Sher, A. (1988) *J. Exp. Med.* **168,** 1675–1684.
5. Street, N. E., Schumacher, J. H., Fong, T. A. T., Bass, J., Fiorentino, D. F., Leverah, J. A. & Mosmann, T. R. (1990) *J. Immunol.* **144,** 1629–1639.
6. Cher, D. J. & Mosmann, T. R. (1987) *J. Immunol.* **138,** 3688–3694.
7. Stout, R. D. & Bottomly, K. (1989) *J. Immunol.* **142,** 760–765.
8. Boom, W. H., Liano, D. & Abbas, A. K. (1988) *J. Exp. Med.* **167,** 1350–1363.
9. Coffman, R. L. & Carty, J. (1986) *J. Immunol.* **136,** 949–954.
10. Stevens, T. L., Bossie, A., Sanders, V. M., Fernandez-Botran, R., Coffman, R. L., Mosmann, T. R. & Vitetta, E. S. (1988) *Nature (London)* **334,** 255–258.
11. Snapper, C. M. & Paul, W. E. (1987) *Science* **236,** 944–947.
12. Sadick, M. D., Heinzel, F. P., Holaday, B. J., Pu, R. T., Dawkins, R. S. & Locksley, R. M. (1990) *J. Exp. Med.* **171,** 115–127.
13. Pearce, E. J., Caspar, P., Grzych, J.-M., Lewis, F. A. & Sher, A. (1991) *J. Exp. Med.* **173,** 159–166.
14. Swain, S. L., Weinberg, A. D., English, M. & Huston, G. (1990) *J. Immunol.* **145,** 3796–3806.
15. Le Gros, G., Ben-Sasson, S. Z., Seder, R. A., Finkelman, F. D. & Paul, W. E. (1990) *J. Exp. Med.* **172,** 921–929.
16. Betz, M. & Fox, B. S. (1990) *J. Immunol.* **145,** 1046–1052.
17. Swain, S. L., Huston, G., Tonkonogy, S. & Weinberg, A. D. (1991) *J. Immunol.* **147,** 2991–3000.
18. Gajewski, T. F. & Fitch, F. W. (1988) *J. Immunol.* **140,** 4245–4252.
19. Gajewski, T. F., Schell, S. R., Nau, G. & Fitch, F. W. (1989) *Immunol. Rev.* **111,** 79–110.
20. Belosevic, M., Finbloom, D. S., Van der Meide, P. H., Slayter, M. V. & Nacy, C. A. (1989) *J. Immunol.* **143,** 266–274.
21. Fiorentino, D. F., Zlotnik, A., Vieira, P., Mosmann, T. R., Howard, M., Moore, K. W. & O'Garra, A. (1991) *J. Immunol.* **146,** 3444–3451.
22. Fiorentino, D. F., Bond, M. W. & Mosmann, T. R. (1989) *J. Exp. Med.* **170,** 2081–2095.
23. Murphy, K. M., Heimberger, A. B. & Loh, D. Y. (1990) *Science* **250,** 1720–1723.
24. Kappler, J. W., Skidmore, B., White, J. & Marrack, P. (1981) *J. Exp. Med.* **153,** 1198–1214.
25. Marrack, P., Shimonkevitz, R., Hannum, C., Haskins, K. & Kappler, J. W. (1983) *J. Exp. Med.* **158,** 1635–1646.
26. Sarmiento, M., Glasebrook, A. L. & Fitch, F. W. (1980) *J. Immunol.* **125,** 2665–2672.
27. Glimcher, L. H., Sharrow, S. O. & Paul, W. E. (1983) *J. Exp. Med.* **158,** 1573–1588.
28. Moore, K. W., Vieira, P., Fiorentino, D. F., Trounstine, M. L., Khan, T. A. & Mosmann, T. R. (1990) *Science* **248,** 1230–1234.
29. Mosmann, T. R., Schumacher, J. H., Fiorentino, D. F., Leverah, J. A., Moore, K. W. & Bond, M. W. (1990) *J. Immunol.* **145,** 2938–2945.
30. Ohara, J. & Paul, W. E. (1985) *Nature (London)* **315,** 333–336.
31. Buchmeier, N. A. & Schreiber, R. D. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 7404–7408.
32. Schumacher, J. H., O'Garra, A., Shrader, B., van Kimmenade, A., Bond, M. W., Mosmann, T. R. & Coffman, R. L. (1988) *J. Immunol.* **141,** 1576–1581.
33. Ernst, D. N., Hobbs, M. V., Torbett, B. E., Glasebrook, A. L., Rehse, M. A., Bottomly, K., Hayakawa, K., Hardy, R. R. & Weigle, W. O. (1990) *J. Immunol.* **145,** 1295–1302.
34. Weinberg, A. D., English, M. & Swain, S. L. (1990) *J. Immunol.* **144,** 1800–1807.
35. Ehlers, S. & Smith, K. A. (1991) *J. Exp. Med.* **173,** 25–36.
36. Maliszewski, C. R., Sato, T. A., Vanden Bos, T., Waugh, S., Dower, S. K., Slack, J., Beckmann, M. P. & Grabstein, K. H. (1990) *J. Immunol.* **144,** 3028–3033.
37. Weaver, C. T., Hawrylowicz, C. M. & Unanue, E. R. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 8181–8185.
38. Pfeiffer, C., Murray, J., Madri, J. & Bottomly, K. (1991) *Immunol. Rev.* **123,** 65–84.
39. Chang, T.-L., Shea, C. M., Urioste, S., Thompson, R. C., Boom, W. H. & Abbas, A. K. (1990) *J. Immunol.* **145,** 2803–2808.
40. Gajewski, T. F., Pinnas, M., Wong, T. & Fitch, F. W. (1991) *J. Immunol.* **146,** 1750–1758.
41. Weaver, C. T., Duncan, L. M. & Unanue, E. R. (1989) *J. Immunol.* **142,** 3469–3476.
42. Seder, R. A., Paul, W. E., Dvorak, A. M., Sharkis, S. J., Kagey-Sobotka, A., Niv, Y., Finkelman, F. D., Barbieri, S. A., Galli, S. J. & Plaut, M. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 2835–2839.
43. Fiorentino, D. F., Zlotnik, A., Mosmann, T. R., Howard, M. & O'Garra, A. (1991) *J. Immunol.* **147,** 3815–3822.
44. O'Garra, A., Stapleton, G., Dhar, V., Pearce, M., Schumacher, J., Rugo, H., Barbis, D., Stall, A., Cupp, J., Moore, K. W., Vieira, P., Mosmann, T. R., Whitmore, A., Arnold, L., Haughton, G. & Howard, M. (1990) *Int. Immunol.* **2,** 821–832.
45. Linsley, P. S., Brady, W., Grosmaire, L., Aruffo, A., Damle, N. K. & Ledbetter, J. A. (1991) *J. Exp. Med.* **173,** 721–730.
46. Freeman, G. J., Gray, G. S., Gimmi, C. D., Lombard, D. B., Zhou, L.-J., White, M., Fingeroth, J. D., Gribben, J. G. & Nadler, L. M. (1991) *J. Exp. Med.* **174,** 625–631.
47. Coffman, R. L., Varkila, K., Scott, P. & Chatelain, R. (1991) *Immunol. Rev.* **123,** 189–207.