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Helminth Antigens Selectively Differentiate Unsensitized CD45RA⁺CD4⁺ Human T Cells in Vitro

Cathy Steel¹ and Thomas B. Nutman

Human filarial helminth infections are characterized by type 2 immune responses to parasite Ag that can persist for the life of the individual; one possible cause for this may be prenatal exposure to the blood-borne microfilarial (Mf) stage of the parasite. To examine the relationship between early exposure to filarial Ag and subsequent immune responsiveness, CD45RA⁺CD4⁺ cells from normal unsensitized donors were stimulated in vitro with soluble microfilarial Ag (MfAg) from the filarial parasite Brugia malayi in the presence of APCs. MfAg alone induced proliferation and IFN-γ and IL-5 production in unsensitized CD45RA⁺CD4⁺ cells, demonstrating the ability of filarial Ags to prime naive T cells in the absence of exogenous cytokines and dendritic cells. Adding exogenous cytokine(s) (particularly IL-12 and IL-4) during priming was able to alter the MfAg-specific responses of CD45RA⁺CD4⁺ cells as well as subsequent responses to Ag. Interestingly, priming solely with MfAg led to enhanced IL-5 production following Ag restimulation, suggesting that MfAg preferentially primes for type 2 responses. These data demonstrate that filarial Ags by themselves can specifically prime CD45RA⁺CD4⁺ cells in vitro and do so in such a way as to deviate the immune response. *The Journal of Immunology*, 1998, 160: 351–360.

![Image of a page from a document](https://example.com/image.png)
CD8 (B9.8; B. Malissen, CIML, Marseilles, France), CD14 (63D3; American Type Culture Collection, Rockville, MD), CD16 (3G8; J. Jaffe, Hahnemann University, Philadelphia, PA), CD19 (FMC63; H. Zola, Woman’s and Children’s Hospital, Adelaide, Australia), HLA-DR (IVA12; American Type Culture Collection), CD56 (PharMingen, San Diego, CA), glycothym (10F7, American Type Culture Collection), and CD45RO (UCHL1; P. Beverly, University College, London, U.K.). Cells were then washed three times in selection buffer, resuspended with goat anti-mouse IgG magnetic beads (PerSeptive Diagnostics, Cambridge, MA) at 50 beads/cell, and again rocked at 4°C for 30 min. Beads were removed twice by magnetic separation, and nonselected cells were washed and counted. These cells were combined with goat anti-mouse IgG magnetic beads (Dynal, Nord, Norway) and five beads per cell for an additional 30 min at 4°C followed by two magnetic separations. Following the two negative selections, approximately 1 to 3% of the initial PBMCs were recovered as CD45RA CD4+ cells. This remaining population was approximately 99% to 100% CD45RA+ T cells as measured by flow cytometry, with 2 to 3% of cells being CD45RA+ RO+.

To phenotype cells, Ab reagents used for further analysis were FITC-conjugated CD45RA and phycoerythrin (PE)-conjugated CD45RO (Immunotech, Westbrook, ME), FITC- and PE-conjugated CD3 (Immunotech), and FITC- and PE-conjugated mouse IgG1 (CalTag, San Francisco, CA) as isotype controls.

Preparation of MfAg

A saline extract of the microfilarial stage of the parasite Brugia malayi (MfAg) was prepared as previously described (21). Briefly, Mf were collected by peritoneal lavage of infected jirds and separated from peritoneal cells on a Percoll discontinuous density centrifugation. The Mf were then washed repeatedly in RPMI with antibiotics and cultured overnight at 37°C in 5% CO2. Worms were harvested the following day, washed with PBS, and frozen at −20°C. The frozen Mf were pulverized, sonicated, and extracted in PBS at 37°C for 4 h and then at 4°C overnight. Following centrifugation at 20,000 × g for 30 min, the supernatant was passed through a 0.45-μm pore size filter and stored in aliquots at −70°C.

Primary Ag stimulation of the CD45RA+ T cell population

CD45RA+ T cells were placed in culture at 1 × 105/ml in C-RPMI and 10% FCS in a 48-well flat-bottom tissue culture plate (Costar, Cambridge, MA) with 25% irradiated (3000 rad; 13C source; Nordion, Kanata, Ontario, Canada) APCs from the B cell/monocyte layer (T cell depleted; ~1% CD3-, 4% CD56-). Cells were cultured in medium alone or with MfAg at 1, 5, or 10 μg/ml with or without recombinant human IL-2 (Cetus, Emeryville, CA) at 25 U/ml, IL-4 (Immunex, Seattle, WA) at 20 ng/ml, IL-10 (Genzyme, Cambridge, MA) at 50 U/ml, or IL-12 (Genetics Institute, Cambridge, MA) at 50 U/ml for 5 days at 37°C in 5% CO2, at which time supernatants were harvested for quantification of cytokine production. All reagents used for these and subsequent assays were free of endotoxin.

Restimulation of T cells with MfAg

In some experiments, cells were rested for 3 days with fresh medium with or without IL-2 at 50 U/ml. Debris and nonviable cells were removed by layering over FCS and spinning at 800 rpm. Viable cells were then counted by trypan blue exclusion and recultured at 1 × 105/ml with 25% APCs and 10 μg/ml of MfAg. In some experiments, IL-2 was also added at 25 U/ml.

Proliferation and limiting dilution analysis

For measurement of proliferation, purified CD45RA+ T cells were cultured with 25% irradiated APCs in 96-well U-bottom plates (Costar) at 1 × 104/ml and C-RPMI supplemented with 10% AB serum (BioWhittaker) with or without MfAg for 5 days at 37°C in 5% CO2, after which 1 μCi/well of [3H]TdR (New England Nuclear Corp., Boston, MA) was added. Cultures were harvested 18 h later, and results were expressed as stimulation indexes (SI = mean counts per minute of stimulated cultures/counts per minute of unstimulated (medium alone) cultures). For limiting dilution analyses, cells were cultured at varying concentrations in a similar manner. The frequency of Ag-specific cells was calculated by conventional means according to the method of Lefkovits and Waldman (22) as modified by Taswell (23). The percentage of responsive wells was based on the number of wells (n = 18 at each cell dilution) with Ag, or Ag with cytokines having proliferation values greater than the mean + 3 SDs of the wells without Ag (n = 6).

Quantification of cytokines by ELISA

For measurement of IL-5, round-bottom plates (Immulon 2, Dynatech, Chantilly, VA) were coated with rat anti-mouse-IL-5 (TRFK5; DNAX, Palo Alto, CA) at 1 μg/ml in PBS followed by washing with PBS and 0.05% Tween. Plates were then blocked with PBS, 5% bovine albumin (Sigma), and 0.05% Tween for 1 h and washed again. Culture supernatants were added and incubated overnight at 4°C; plates were then washed, and IL-5 was detected with nitroiodophenyl (Sigma)-conjugated rat anti-human IL-5 (JES1-5A10; PharMingen) in PBS, 1% bovine albumin, and 0.05% Tween-20 (ELISA diluent) for 2 h at 37°C. Following washing, an antinitrophenyl peroxidase-conjugated Ab (J4-HRP; DNA) in ELISA diluent was added for 1 h at 37°C. Plates were again washed and subsequently developed with a peroxidase substrate system (ABTS; Kirkegaard and Perry, Gaithersburg, MD); colorimetric development was detected at 450 nm using a microplate reader (Molecular Devices, Sunnyvale, CA). Measurements of IFN-γ and IL-10 were performed similarly using the following Abs: anti-human IFN-γ (Endogen, Woburn, MA) or anti-human IL-10 (JES3–9D7; PharMingen) for coating, polyclonal rabbit anti-human IFN-γ (author’s laboratory) or biotin-conjugated anti-human IL-10 (JES3–12G8; PharMingen) for detection, and peroxidase-conjugated goat anti-rabbit IgG or alkaline phosphatase-conjugated streptavidin (Jackson ImmunoResearch Laboratories, West Grove, PA) for enzyme conjugation. Quantification was based on standardized curves using recombinant cytokines.

Statistical analyses

Because the data were generally not normally distributed, the Wilcoxon signed rank test was used for comparison of data.

Results

Primary responses: proliferation

CD45RA+ T cells were capable of responding to MfAg at 10 and 5 μg/ml, with SI values ranging from 2.0 to 8.5 (data not shown); there was little proliferative response at 1 μg/ml, even with the addition of IL-2 at 50 U/ml. In the absence of exogenous cytokines, the number of Ag-responsive CD45RA+ cells ranged from 1/52,000 to 1/87,000 at an Ag concentration of 10 μg/ml (Fig. 1). The frequency increased when exogenous IL-2 and IL-4 were added in addition to Ag (Fig. 1B).

To exclude the possibility that MfAg was acting as a superantigen, CD45RA+ T cells and unselected T cells (all CD3+) from the same individuals were stimulated with either MfAg or the superantigen Staphylococcus enterotoxin B (SEB) at various concentrations in the presence of irradiated or paraformaldehyde-fixed autologous APCs. While both CD45RA+ and total CD3+ T cells responded to MfAg using normal irradiated APCs, neither T cell population proliferated in response to Ag in the presence of fixed APCs, demonstrating that MfAg requires processing to induce T cell activation and proliferation (data not shown). Interestingly, while both groups of T cells (unselected and CD45RA+) responded to SEB using irradiated autologous APCs, only unselected (all CD3+) T cells were activated by SEB with fixed APCs, indicating that CD45RA+ T cells may have requirements for responses to superantigens that differ from those of more heterogeneous populations of T cells. Further, MfAg was unable to induce proliferation in unselected T cells when mismatched APCs were used (data not shown).

Phenotype changes

The low frequency of MfAg-responsive cells, as demonstrated above by the precursor frequencies, was also evident by the small percentage of cells that exhibited a change in phenotype from CD45RA+ RO− to CD45RA+ RO+. In three individual experiments, two of three individuals showed a demonstrable, but small, shift to the CD45RO+ phenotype following stimulation with MfAg and a 3-day rest period with rIL-2. In the two patients who responded to Ag with a phenotypic change, an average of 82% of cells retained the CD45RA+ RO− phenotype compared with 86% in those cells stimulated with medium alone. Three percent of cells changed to the CD45RA+ RO+ phenotype compared with 1% of cells in medium. In addition, 6% of cells demonstrated a shift to
the double-positive CD45RA<sup>+</sup>RO<sup>bright</sup> phenotype compared with 3% in cells stimulated with media alone.

In a single individual in whom there were sufficient numbers of cells to be restimulated for 5 days with MfAg, 29% of cells exhibited the CD45RA<sup>+</sup>RO<sup>+</sup> phenotype, with a corresponding loss of 32% of CD45RA<sup>+</sup>RO<sup>+</sup> cells. Repeated stimulation with MfAg eventually caused a complete shift to the CD45RA<sup>+</sup>RO<sup>+</sup> phenotype (data not shown).

**Primary cytokine responses**

MfAg by itself induced low levels of IFN-γ (geometric mean net production = 5.8 pg/mL; range = 0–250) and IL-5 (1.8 pg/mL; range = 0–63.4) in 7 of the 11 individuals studied (data not shown). Priming with MfAg in the presence of exogenous IL-2 caused an increase in the production of IFN-γ by the CD45RA<sup>+</sup> T cells in 8 of 11 individuals (geometric mean = 868.3 pg/mL) over that produced in response to IL-2 alone (geometric mean = 639.1 pg/mL; P = 0.041; Fig. 2). In contrast, IL-5 production (used as a prototypical type 2 response indicator) was variable, increasing in 6 of 11 individuals when primed with both Ag and IL-2 (geometric mean = 13.8; with IL-2 alone, 8.7 pg/mL; P = 0.086).

The effect of the cytokine milieu present at the time of priming with Ag was then assessed by comparing the production of IFN-γ and IL-5 in response to MfAg with IL-2, IL-4, or the combination...
of IL-2 and IL-4 to the production in the presence of these cytokines alone (Fig. 3). While MfAg increased production of IFN-γ in 8 of 11 individuals (same individuals as in Fig. 2) in the presence of exogenous IL-2 (median = 277 pg/ml), when IL-4 was present either alone or in combination with IL-2, IFN-γ production was absent or diminished. This suggests that IL-4 predominates over IL-2 if present at the time of priming with Ag. IL-5 production was enhanced by MfAg, particularly if IL-4 was also present (Fig. 3). As seen, in the presence of IL-2 at priming, MfAg enhanced IL-5 production in 6 of 11 individuals (same individuals as in Fig. 2; median = 8.2 pg/ml); 7 of 9 individuals showed increased IL-5 production in the presence of IL-4 (median = 18.7 pg/ml; P = 0.018), and 7 of 10 showed increased production when IL-4 was used in conjunction with IL-2 (median = 13.4 pg/ml; P = 0.066).

**IL-10 priming and production**

Because filarial Ags and, in particular, the Mf and infective larval stage (L3) Ags induce in vitro IL-10 secretion in naturally infected individuals, and because this IL-10 induction may influence a patient’s response upon subsequent exposure to Ag, CD45RA⁺ T cells were primed in the presence of MfAg with or without IL-10 in the presence or the absence of IL-2 or IL-4 (Fig. 4). Priming in the presence of IL-10 greatly reduced the production of both IFN-γ and IL-5 compared with that seen in response to MfAg alone and in response to Ag plus IL-2 or IL-4. Although the mechanism for this down-regulation of cytokine production by IL-10 was not specifically addressed here, toxicity did not appear to be the reason for this decrease in production, as cell number and viability were no different in cultures containing IL-10 from those in cultures without IL-10.

Although production of IL-10 by CD45RA⁺ naive T cells could not be separated from that produced by the APCs in these experiments, IL-10 production in response to MfAg under the various priming conditions was nevertheless examined (Table I). Unexpectedly, primary stimulation of the CD45RA⁺ cells by MfAg induced a down-regulation of IL-10 in five of six individuals compared with the production by cells in medium alone; however, this difference was not significant (P = 0.12).

The addition of IL-2 during priming with MfAg, however, did increase IL-10 production over that produced by cells stimulated with IL-2 alone (P = 0.04; Table I). No differences, however, were observed when IL-4 and MfAg were used in priming compared with priming with IL-4 alone (P = 0.72). When both IL-2 and IL-4 were present at priming in concert with MfAg, four of five individuals demonstrated an increase in IL-10 production over that in response to the combination of cytokines alone, although the levels were not significantly different (P = 0.08).

**Priming with IL-12**

In three individual experiments, the use of IL-12 at priming enhanced IFN-γ production; this production was augmented in two of three individuals with the addition of MfAg (Fig. 5). When IL-2 was added to IL-12, the levels of IFN-γ increased over those produced in response to IL-12 alone and again were enhanced in two

![FIGURE 3. Net production of IFN-γ (left panel) and IL-5 (right panel) by CD45RA⁺ T cells to MfAg in the presence of cytokines during priming. Net production was calculated as the production of IFN-γ and IL-5 in response to MfAg in the presence of cytokines minus the production in the presence of cytokine(s) alone. Each dot represents one individual, and the shaded area represents the level at which there were either no differences or diminished production in the absence of Ag compared with that in the presence of Ag.](http://www.jimmunol.org/)
of three individuals with the addition of MfAg. There was virtually no IL-5 production in the presence of IL-12 priming, and the addition of IL-2 did not change this.

IL-4, as shown in previous experiments, however, was capable of inducing IL-5 in all three individuals (shown with the addition of IL-12; Fig. 5, bottom panel). In two of three individuals, this production was again enhanced with the addition of MfAg. There was very little IFN-\(\gamma\) production in the presence of IL-4, despite the addition of either IL-2 or IL-2 plus MfAg. When IL-4 was used in combination with IL-12, IFN-\(\gamma\) production increased in both individuals tested. Interestingly and unexpectedly, however, when priming was performed in the presence of both IL-4 and IL-12 with MfAg, the highest levels of IL-5 were produced.

Secondary cytokine responses

CD45RA\(^+\)CD4\(^+\) T cells originally primed with MfAg alone or in medium alone were rested for 3 days and then restimulated with MfAg. When cells had been primed with MfAg, the subsequent IFN-\(\gamma\) production in the secondary cultures was increased in only one of seven individuals from cells primed in medium alone (Fig. 6). In contrast, IL-5 production increased in five of seven individuals, indicating that cells primed in the presence of filarial Ag alone, without any exogenous cytokine, deviate toward a type 2 phenotype on restimulation with the Ag. This finding occurred regardless of exogenous IL-2 being added during the secondary stimulation (not shown).

The pattern of secondary cytokine production differed depending on the nature of the cytokine milieu present at the time of CD45RA\(^+\) cell priming. Thus, if Ag priming occurred in the presence of IL-2, IFN-\(\gamma\) production increased in three of five individuals (Fig. 7). In contrast, there was little IL-5 production under these same priming conditions, with IL-5 production in four of five individuals either decreasing or showing no change.

In four individual experiments in which IL-4 was present at priming with Ag, IFN-\(\gamma\) decreased in all individuals compared with production by those cells primed with IL-4 alone (Fig. 7). In contrast, IL-5 levels were higher when cells were primed with Ag in the presence of IL-4 than when they were primed with Ag in the presence of IL-2. Interestingly, priming in the presence of IL-4 alone, without Ag, produced significantly higher IL-5 production (\(P = 0.043\)) upon contact with Ag than did priming in the presence of IL-2 alone.

Discussion

A system for in vitro priming of CD45RA\(^+\)CD4\(^+\) naive human T cells by soluble helminth Ag has been developed and used to demonstrate that these Ags selectively differentiate CD4\(^+\)CD45RA\(^+\) cells. To date, it has been difficult to prime naive T cells with soluble Ag, with most previous in vitro studies having used direct stimulation with anti-CD3 plus a second signal (such as those delivered through anti-CD28 (24, 25), anti-CD70 (26), CD32-transfected fibroblasts (27, 28), mitogens (29, 30), or various exogenous cytokines (15, 31–34)) or by using cell-bound alloantigen (35–38).
or bacterial superantigens (39, 40). Alternatively, the study of Ag-induced differentiation of naive cells has been accomplished using TCR transgenic mice (37, 41–44). We have demonstrated that using a soluble parasite Ag alone in the presence of APCs, highly purified naive T cells from unsensitized normal human donors were able to both proliferate and secrete type 1- and type 2-associated cytokines. In addition, these cells continued to respond to secondary stimulation with Ag.

Naive T cells responded to MfAg in a dose-dependent fashion without the use of exogenous cytokines; the very low frequencies of responsive cells, as demonstrated by the phenotypic changes and precursor frequencies, indicates that this probably was not a recall response to cross-reactive Ags. Additionally, although the MfAg preparation consists of a crude mixture of proteins, the Ag preparation did not appear to have any superantigen or mitogenic effects, as the responses required processing by autologous APCs (data not shown).

Of interest, priming could be accomplished in the presence of primarily B cells and monocytes rather than dendritic cells, a situation not paralleled in murine systems in which B cells have been shown to be unable to prime naive T cells in vivo, although they were able to present Ag to activated T cells (45). In contrast, cultured Langerhans cells have been shown to be capable of priming naive T cells to soluble Ag (35) and, unlike B cells or monocytes, were also successful in inducing significant primary in vitro stimulation of hapten-specific T cells (46). Further, it has been suggested that naive cells primed in the presence of unprimed B cells, but not dendritic cells, should lead to tolerance (47), a hypothesis not supported by the present study using human cells.

Mehta-Damani and colleagues have been able to generate Ag-specific human T cell lines from naive CD4\(^+\) cells, but only when dendritic cells were used as APCs during the priming; macrophages were unable to elicit a response by T cells regardless of the number of APCs used (48). Since the number of APCs used in our study was similar in range to that used by Mehta-Damani, and since macrophages are considered better Ag presenters than either B cells or monocytes, the fact that we were able to generate a response in naive cells may be related to the nature of the Ag used. In two studies in mice, for example, dendritic cells were shown to be more efficient at presenting peptides to naive cells, whereas B cells were necessary for priming to the protein forms of the same Ags (49, 50). Whatever the mechanism, MfAg is able, as a soluble extract, to prime cells under the conditions used in this study. Indeed, in preliminary experiments, several other helminth Ags (e.g., *Toxocara*, *Schistosoma*, and *Onchocerca*) were also able to prime CD45RA\(^-\)CD4\(^+\) T cells to produce both IL-5 and IFN-\(\gamma\), suggesting that helminth Ags will be particularly effective in eliciting responses from naive T cells (data not shown). Of note, soluble adult *Brugia* male Ag (from a stage devoid of Mf), while capable of priming for both IFN-\(\gamma\) and IL-5, induced IFN-\(\gamma\) at levels 6 to 100 times that produced by MfAg. A relatively high level of IFN-\(\gamma\) was also observed in a single experiment using a soluble extract of the nonhelminth intracellular parasite *Toxoplasma gondii*.
While MfAg alone was able to elicit a response in most individuals, as illustrated by both Ag-specific proliferation and cytokine production, the addition of exogenous cytokines to the cultures clearly altered the nature of the response. It has been shown from many studies that the cytokine milieu, particularly at the time of priming, influences the nature of the subsequent immune response (10–17, 33, 51). In the present study, for example, when exogenous IL-2 was used in combination with MfAg, the production of IFN-γ was enhanced in nearly all individuals, whereas IL-4 favored the production of IL-5.

IL-4, like IL-2, has long been considered a T cell growth factor; however, its potential polarizing effect with regard to CD4⁺ T cell differentiation has been more recently elucidated. While IL-2 has been shown to induce both type 1 and type 2 cytokines (16), the presence of IL-4 favors the type 2 cells (14, 18, 41) and appears to down-regulate IFN-γ (16, 51, 52). From our data, the effect of IL-4, either alone or in combination with MfAg or IL-2, on the development of CD45RA⁺ cells was apparent through analysis of both primary and secondary IFN-γ and IL-5 production (Figs. 3 and 7). The addition of IL-4 exogenously with Ag strongly diminished primary IFN-γ production and enhanced IL-5 production even when IL-2 was present at priming. This IFN-γ down-regulation continued upon restimulation with Ag, even though IL-4 was not used in the secondary stimulation. It has been theorized, at least from data observed using transgenic mice, that the effect of IL-4 occurs early in the activation and priming stages and has a lesser effect on activated cells (52); in contrast, it has also been demonstrated that in human neonatal cells, while the induction of the type 1 phenotype appears to be stable, induction of a type 2 phenotype requires more constant exposure to IL-4 (29, 53). Our data suggest that the presence of MfAg perhaps stabilizes the type 2 response not only by inducing IL-4 but also by diminishing IFN-γ. Thus, having a sustained source of IL-4 skews the cellular response toward a type 2 (and away from a type 1) response, a finding given credence by a study in mice demonstrating that sustained exposure to IL-4 through diffusion chambers enhances IL-4 production while simultaneously decreasing IFN-γ production (13).

Similar to results obtained in this investigation, several studies have indicated that exogenous IL-4 at the time of priming leads to diminished IFN-γ production and/or the production of increased levels of IL-4 or IL-5 (13, 16, 18, 41, 51, 52). Other studies, however, have shown that the addition of IL-4, either alone (32) or in combination with neutralizing anti-IL-2 Ab (17), is not sufficient for the induction of IL-4. Because IL-5 (rather than IL-4) was used as the type 2 indicator cytokine, and because IL-4 and IL-5 may be regulated independently (54), this might explain the differences between the findings in the present study and those of other studies. Our data do indicate, however, that IL-2 and IL-4 together have an additive effect, particularly in the diminution of IFN-γ production.

IL-12, like IL-4, has been implicated in the differentiation of naive T cells into mature effector CD4⁺ T cells. Most notable is the finding of enhanced IFN-γ production when cells are primed with IL-12 (14, 31, 51, 55), although not all this IFN-γ production is IL-12 dependent (56). The findings from this study also demonstrated that priming in the presence of exogenous IL-12 leads not only to enhanced IFN-γ but also to decreased levels of IL-5. It was apparent that IL-2 worked synergistically with IL-12 to polarize the response toward a type 1 phenotype. Whether this was
due to increased numbers of cells producing IFN-γ or to increased production within a single cell has not yet been determined, although the latter is less likely.

Most unexpected was the observation of extremely high production of IL-5 when T cells were primed in the presence of IL-4 together with IL-12, production that was further enhanced with soluble MfAg. Indeed, the level of IL-5 produced under these conditions surpassed that seen in all other priming conditions studied. Since IL-12 by itself normally down-regulates type 2 cytokine production, the mechanism underlying the synergism (with regard to IL-5 production) seen when IL-4 and IL-12 are present together remains to be elucidated. In a human in vitro system that primed CD4^+ and CD8^+ T cell clones for IL-4 production, IL-12 when used in conjunction with IL-4 was shown to induce maximal amounts of IL-4 (57). Further, in a study in unsensitized mice, the use of IL-12 and IL-4 together at priming (in an Ag-independent system) showed increased production of IL-4 (compared with IL-4 alone), but only on restimulation (14). It is conceivable that IL-12 is expanding the type 2 precursors responding to the exogenous IL-4 added, although it is generally thought to only affect type 1 cells in this way. Since IL-4 is normally the dominant cytokine when used with IL-12 (14), it is possible that these naive cells are responding initially to the IL-4 but are then expanding or, alternatively, increasing their individual levels of IL-5 production. It has also been proposed that priming in the presence of IL-4 plus IL-12 selectively expands a small IL-4-producing population of CD3^+ CD4^+ cells (29).

Filarial infections are particularly relevant to the study of CD4^+ subset differentiation. In both lymphatic filariasis and onchocerciasis (the two most common forms of filarial infection), the majority of infected individuals have immune responses that are deviated away from a type 1 response (7, 8) and have, as a result, an inability to clear the parasites such that they are constantly and chronically exposed to Mf and MfAg. This is in contrast to those who have cleared their Mf, whose immune responses are generally mixed (type 1 and type 2). In this study, priming with MfAg alone, without exogenous cytokines, followed by restimulation with Ag enhanced the type 2 response and decreased IFN-γ levels. In utero exposure to MfAgs circulating in the mother’s blood presumably acts in a similar fashion, such that type 2 responses are induced when the subject is subsequently infected naturally later in life.

Because IL-10 has been implicated in the down-regulation of type 1 responses in actively infected individuals (7, 8), the levels of IL-10 produced by CD4^+ CD45RA^-CD4^+ T cells were examined in response to MfAg. Interestingly, these cells had a trend toward lower levels of IL-10 production in response to MfAg, possibly indicating a different means of regulation in unexposed naive individuals than in those with active infection who are constantly exposed to MfAg. Addition of exogenous IL-10 down-regulated...
not only the production of IFN-γ but also that of IL-5 in the presence of Ag, possibly acting globally on Ag presentation. The differentiation to a type 2 phenotype may be dependent upon other factors, including the dosage of Ag (19, 58) and the type of APC (18) used. Further, priming through stimulation of CD28 has been shown to lead to the development of a type 2 phenotype (30), suggesting that stimulation with MfAg in the absence of exogenous cytokines may be acting through CD28 and its ligand(s). Early in utero exposure to filarial parasite Ag can lead to a long-term altered response to the Ag (1), although the mechanisms underlying this phenomenon remain to be determined. A type 2 phenotype has been shown to develop in a small percentage of cells before encountering TCR signals or exposure to IL-4 (25); in addition, cloned human naive T cells appear to have a default development to become IL-4 and IL-5 producers (59). Individuals exposed in utero to MfAg appear in many ways to be hyporesponsive to this stage of the parasite, but not necessarily to the adult or infective stages (1), probably due to early alterations in T cell phenotypes or perhaps to Ag-stimulated apoptosis. To date, no obvious deletions have been noted in the Vβ T cell repertoire in these individuals (C. Steel, unpublished observations). Recently, it has been suggested, based on murine studies, that the development of a type 1 or a type 2 response to Ag is dependent on the mode of immunization during the neonatal period or on a redistribution of memory cells (into the spleen) (60). Nevertheless, the ability to activate unsensitized naive T cells by soluble filarial Ag provides a system to study the long term development of these cells under a variety of priming conditions, thereby providing insight into how early exposure to parasite Ag (or any Ag) can alter subsequent reactivity on a possibly permanent basis.

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References