JCI The Journal of Clinical Investigation

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J Clin Invest. 1993;92(4):1667-1673. https://doi.org/10.1172/JCI116752.

Research Article

The immunological mechanisms involved in maintenance of an asymptomatic microfilaremic state (MF) in patients with lymphatic filariasis remain undefined. MF patients have impaired filarial antigen (Ag)-specific lymphocyte proliferation and decreased frequencies (Fo) of Ag-specific T cells, and yet elevated serum IgE and antifilarial IgG4. To investigate the mechanism of Ag-specific anergy in MF patients in contrast to amicrofilaremic individuals with chronic lymphatic obstruction (CP), the Fo of Ag-specific lymphocytes from peripheral blood mononuclear cells secreting either IL-4 or IFN-gamma were assessed by filter spot enzyme-linked immunosorbent assay, and IL-10 and transforming growth factor-beta (TGF-beta) mRNA transcript levels were assessed by a semiquantitative reverse transcriptase polymerase chain reaction technique. The Fo of filaria-specific IL-4-secreting lymphocytes were equivalent in both MF (geometric mean [GM] = 1:11,700) and CP (GM = 1:29,300 P = 0.08), whereas the Fo of IFN-gamma-secreting lymphocytes were lower in MF (GM = 1:39,300) than in CP (GM = 1:4,200, P < 0.01). When the ratio of IL-4/IFN-gamma (T helper type 2 [Th2]/Th1)-secreting cells was examined, MF subjects showed a predominant Th2 response (8:1) compared with a Th1 response in CP individuals (1:4). mRNA transcript levels of IL-10 were also significantly elevated in MF compared with CP individuals (P < 0.01). Further, IL-10 and TGF-beta were shown to have a role in modulating the Ag-specific anergy among MF subjects, […]

Cytokine Control of Parasite-specific Anergy in Human Lymphatic Filariasis

Preferential Induction of a Regulatory T Helper Type 2 Lymphocyte Subset

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Abstract

The immunological mechanisms involved in maintenance of an asymptomatic microfilaremic state (MF) in patients with lymphatic filariasis remain undefined. MF patients have impaired filarial antigen (Ag)-specific lymphocyte proliferation and decreased frequencies (Fo) of Ag-specific T cells, and yet elevated serum IgE and antifilarial IgG4. To investigate the mechanism of Ag-specific anergy in MF patients in contrast to amicrofilaremic individuals with chronic lymphatic obstruction (CP), the Fo of Ag-specific lymphocytes from peripheral blood mononuclear cells secreting either IL-4 or IFN-γ were assessed by filter spot enzyme-linked immunosorbent assay, and IL-10 and transforming growth factor-β (TGF-β) mRNA transcript levels were assessed by a semiquantitative reverse transcriptase polymerase chain reaction technique. The Fo of filaria-specific IL-4-secreting lymphocytes were equivalent in both MF (geometric mean [GM] = 1:11,700) and CP (GM = 1:29,300 $P = 0.08$), whereas the Fo of IFN-γ-secreting lymphocytes were lower in MF (GM = 1:39,300) than in CP (GM = 1:4,200, $P < 0.01$). When the ratio of IL-4/IFN-γ (T helper type 2 [Th2]/Th1)-secreting cells was examined, MF subjects showed a predominant Th2 response (8:1) compared with a Th1 response in CP individuals (1:4). mRNA transcript levels of IL-10 were also significantly elevated in MF compared with CP individuals ($P < 0.01$). Further, IL-10 and TGF-β were shown to have a role in modulating the Ag-specific anergy among MF subjects, in that neutralizing anti-IL-10 or anti-TGF-β significantly enhanced lymphocyte proliferation response (by 220–1,300%) to filarial Ags in MF individuals. These findings demonstrate that MF subjects respond to parasite antigen by producing a set of suppressive cytokines that may facilitate persistence of the parasite within humans while producing little clinical disease. (*J. Clin. Invest.* 1993. 92:1667–1673.) Key words: lymphatic filariasis • tolerance • T helper subsets • IL-10 • ELISPOT

Introduction

The human lymphatic filariases, estimated to affect ~ 100 million people worldwide, produce a range of host immune responses that have been implicated in the pathogenesis of the different clinical manifestations of this infection (1). Host reactions to both brugian and bancroftian filariasis infections range from those of asymptomatic individuals with circulating microfilariae to others who exhibit chronic lymphedema and occasionally pulmonary symptoms (tropical pulmonary eosinophilia) but have no circulating microfilariae (2). This breadth of host reaction to infection may reflect the nature of different individuals' immune response to filarial infection (2, 3). For example, asymptomatic microfilaremia (MF)[1] is thought to result from parasite-specific "immune hyporesponsiveness" by the host, thereby allowing the microfilariae to persist in the circulation (3–5). This hypothesis has been supported by observations of selectively impaired T and B cell responses to filarial antigens (Ags) among MF subjects (3–11), but the Ag-specific anergy does not extend to all immune responses in MF individuals, as frequencies of polyclonal IgE- and IgG4-secreting lymphocytes (12) and serum antifilarial IgE and IgG4 levels are usually elevated (9, 13). In contrast, strong filarial Ag-driven T and B cell responses are observed among amicrofilaremic individuals with chronic lymphatic obstruction (CP).

The mechanisms underlying the apparent immunological hyporeactivity in MF patients are poorly understood. Previous studies in patients with brugian filariasis have suggested active suppression by either monocytes (8) or suppressor $CD8^+$ T lymphocytes (7). Similar studies with PBMC from persons with *Wuchereria bancrofti* infection have failed to identify such suppressor cell populations (11, 14). Furthermore, the observation that PBMC from MF patients with bancroftian filariasis did not produce parasite-specific antibody in response to either parasite Ag or the T cell–dependent B cell mitogen, PWM (14), suggested that MF subjects may, in fact, lack Ag-responsive T cells. Indeed, we recently reported that MF subjects appear to have significantly reduced frequencies (Fo) of filarial Ag-specific T cells, estimated by limiting dilution analysis, compared with individuals with CP that approached that of normal uninfected individuals (12). However, in the limiting dilution analysis, we observed that cultures with fewer lymphocytes often had similar, and sometimes even greater, numbers of cells capable of proliferating to filarial Ags than did cultures seeded with larger numbers of T cells. This observation suggested that several interacting populations of Ag-reactive $CD4^+$ cells might exist and could modulate the response of other cells to parasite antigens. In addition, these studies highlighted a limitation of standard limiting dilution analysis since lymphocyte proliferation represents a late T cell response that may be influenced by cross-regulatory molecules such as IL-10, TGF-β, or IFN-γ (15–17). Furthermore, T cells may secrete

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Received for publication 30 November 1992 and in revised form 11 May 1993.

1. *Abbreviations used in this paper:* Ag, antigen; BmA, *Brugia malayi* filarial antigen; CP, chronic lymphatic obstruction; ELISPOT, filter spot-ELISA; Fo, frequencies; MF, microfilaremia; PPD, purified protein derivative.

cytokines (e.g., IL-4 and IL-5) rather than proliferate strongly in response to antigens. Thus, the present study evaluated T cell responses to parasite Ags by analysis of cytokine production by individual peripheral blood–derived lymphocytes in filarial infected individuals.

In the current study we therefore determined: (*a*) whether MF subjects had lymphocytes capable of responding to parasite Ag, as determined by cytokine secretion of individual T cells using a filter immunoplaque assay; (*b*) whether different patterns of cytokine production (e.g., IFN-γ vs. IL-4) are produced by individual lymphocytes from MF vs. CP subjects; and (*c*) whether the "impaired" T cell proliferation characteristically seen in MF individuals results from active suppression by the production of additional cytokines such as IL-10 or TGF-β.

Methods

Study population. 29 adult volunteers living in the area of Madras, India, where bancroftian filariasis is endemic, were studied. Clinical history, physical examination, and PBMC were obtained from each study participant. Microfilariae were enumerated by filtration of 3 ml of nocturnally collected blood through a polycarbonate filter (Nuclepore Co., Pleasanton, CA) (18). Additionally, five normal North American individuals (three females and two males) between 30 and 41 yr of age were studied as controls. Not all patients were studied with all of the assays. Selection was based only on the availability of patients for study.

Antigens and mitogens. Brugia malayi filarial antigen (BmA) was prepared as the saline extract of adult-stage parasites (9). PWM (GIBCO BRL, Gaithersburg, MD) and the mycobacterial Ag, purified protein derivative (PPD) (Connaught Labs, Willowdate, Ontario, Canada), were used in parallel cultures.

Isolation of PBMC. All studies were performed on fresh PBMC separated by Ficoll-diatrizoate gradient centrifugation (19) from heparinized venous blood.

ELISA for parasite-specific IgG and IgE. The ELISA for parasite-specific IgG and a solid-phase RIA for IgE were performed with BmA antigen exactly as described previously (14, 20).

Filter spot-ELISA (ELISPOT). ELISPOTs to determine the frequency of specific cytokine-producing lymphocytes were performed as described previously (21). For all assays, PBMC were used with the antigens (BmA or PPD) or with a combination of 50 ng/ml phorbol 12-myristate 13-acetate (PMA) and 1 μg/ml ionomycin. These mitogens were used together because they produced maximal IL-4/IL-5 synthesis compared with other mitogenic stimuli tested (our personal observations).

Analysis of cytokine mRNA. PBMC were cultured at 3×10^6 cells/ml in 1-ml cultures. After 12 and 48 h, PBMC were placed directly into RNAzol B (Tel-Test, Friendswood, TX) and stored at −70°C until use. RNA isolation was performed as described (22). Cytokine transcript levels were measured using a modification of a semiquantitative reverse transcriptase PCR technique previously described (15, 23). Briefly 1 μg of total RNA was reversed transcribed using Moloney murine leukemia virus reverse transcriptase (GIBCO BRL) in a 50-μl reaction. The reaction mixture was then diluted 1:4, and 10 μl of diluted product was used for specific amplification of cytokine mRNA using Taq DNA polymerase (Promega Biotec, Madison, WI). PCR products were separated on a 1% agarose gel and transferred to a Hybond N^+ membrane (Amersham International, Amersham, UK) using standard blotting techniques. Southern transfers were subsequently probed with internal cytokine-specific oligonucleotides and visualized using the enhanced chemiluminescence detection system (Amersham International). Autoradiographs were scanned with a scanner (model 600ZS; Microtek, Torrance, CA). PCR reaction conditions were strictly defined for each cytokine such that a log-linear relationship was obtained between the amount of specific cytokine mRNA and the signal density of the probed PCR product in the detection systems. The amount of cytokine-specific mRNA in a sample was determined by comparison of the signal density of the probed PCR product with that of standard curve generated by simultaneous amplification and probing of step-wise dilutions of reverse transcribed product for known amounts of mRNA. To control for the relative amount of products reverse transcribed and to assess the amount of mRNA in each sample, concurrent measurement of mRNA for the constitutively expressed gene hypoxanthine-quanine phosphoribosyl transferase (HPRT) was made. The oligonucleotide sequences for HPRT, IL-10, and TGF-β used have been previously described (24).

Proliferation assays. PBMC (10^6/ml) were cultured in round-bottomed microtiter plates in 0.2 ml of C-RPMI 1640 supplemented with gentamicin (80 μg/ml), Hepes (25 mM), and 10% human AB serum. Media (control), BmA, PPD, or PWM were added to the wells. Cultures were performed in quadruplicate or sextuplicate and maintained in a humidified atmosphere with 5% CO_2 at 37°C. After 6 d, 20 μl of C-RPMI containing 1 μCi [^{3}H]thymidine (New England Nuclear, Boston, MA) was added to the cultures that were then incubated for an additional 16 h before the cells were collected on glass filters with a cell harvester (Skatron Inc., Sterling, VA). Thymidine incorporation was measured by liquid scintillation spectroscopy (Pharmacia LKB Nuclear Inc., Uppsala, Sweden).

Neutralizing anticytokine antibodies. Neutralizing anticytokine antibodies (or appropriate control antibodies) were used as follows: (*a*) anti-IL-10 (JES 3-9D7 [53]) at 10 μg/ml (irrelevant rat mAb of the IgG1 isotype was used as a control); (*b*) anti-TGF-β polyvalent chicken Ab and appropriate control antiserum at 5 μg/ml (AB-101-NA; R&D Systems, Minneapolis, MN); (*c*) anti-IL-2 polyvalent rabbit serum (1:200 dilution) with preimmune sera control; and (*d*) anti-IFN-γ polyvalent rabbit serum (1:200 dilution) with preimmune sera control as previously described (25).

Data analysis. Means were compared using Student's *t* test with logarithmically transformed data. The Fisher's exact test was used to examine the effect of anti-IL-10 and anti-TGF-β Abs on lymphocyte proliferation shown in Fig. 1.

Results

Study subjects. Patients were selected based on their availability and classified into two groups. Asymptomatic individuals with circulating MF and patients without MF but who had a history of lymphangitis or lymphadenitis and evidence of chronic lymphatic obstruction (CP) in one or more limbs were studied. The CP patients were of both sexes and older (median age, 40 yr) than the MF patients (median age, 30 yr). Serum filaria-specific IgG4 and polyclonal IgE levels were markedly elevated in MF individuals and significantly higher than among CP subjects (Table I). In healthy uninfected North American individuals specific IgG4 is undetectable and polyclonal IgE is <450 ng/ml. In contrast, total serum IgG levels were significantly lower among MF vs. CP subjects (Table I). Filarial-specific serum IgG levels (MF, geometric mean [GM] = 442 U/ml; CP, GM = 585 U/ml) and IgE levels (MF, GM = 101 ng/ml; CP, GM = 120 ng/ml) were similar between the two groups.

Lymphocyte proliferation responses to filarial Ags among MF individuals were significantly diminished compared with CP subjects (Table I; $P < 0.05$). This Ag-specific immune "unresponsiveness" in MF individuals was restricted to filarial antigens as the mean PPD-driven T cell proliferation was similar in the two groups (MF, GM stimulation index [S.I.] = 14; CP, GM S.I. = 34; $P = 0.32$).

Enhanced Fo of IL-4 relative to IFN-γ secreting lymphocytes in MF vs. CP subjects. Because T cell proliferation is a late and restricted indicator of T cell behavior, we examined an-

Table I. Patient Population: Clinical Characteristics and Serum Antibody Levels

Diagnosis	Median age	Microfilariae	Filarial-specific IgG4	Polyclonal IgG	Polyclonal IgE	Stimulation indices to BmA
	yr	*no./ml*	*μg/ml*	*mg/ml*	*ng/ml*	
MF (n = 12)	30 (21–39)	414 (78–960)*	48 (13–132)‡	1,300 (940–1,540)§	2,724 (796–7,560)§	2.2 (0.8–6.6)‖
CP (n = 17)	40 (24–65)	0	0.2 (0–71)	2,009 (1,510–2,550)	859 (97–8,200)	48 (3.8–770)

* Geometric means (range). ‡ $P < 0.001$ compared with CP. § $P < 0.01$ compared with CP. ‖ $P < 0.05$ compared with CP.

other measure of immune response to specific antigens, that of cytokine production. Because of the low frequency of antigen-reactive T cells and the ephemeral nature of IL-4 in culture supernatants, cytokine production by individual lymphocytes was examined by ELISPOT. The geometric mean Fo of cells secreting IL-4 in response to BmA (1:11,730) were similar among MF subjects compared to CP individuals (GM = 1:29,400; P = 0.08) (Fig. 1 *A*). In contrast, BmA-driven IFN-γ-secreting lymphocytes were significantly diminished among MF individuals (GM = 1:39,340) relative to CP subjects (GM = 1:4,230; $P < 0.01$) (Fig. 1 *B*). Thus, though MF and CP individuals showed similar Fo of IL-4-secreting cells in response to filarial antigen, the relatively low Fo of IFN-γ-secreting lymphocytes from MF compared with CP subjects indicated that the ratios of Ag-driven IL-4 to IFN-γ-secreting lymphocytes in the PBMC were distinctly different in the two clinical groups. MF patients averaged eightfold greater Fo of IL-4- to IFN-γ-secreting cells (Fig. 1C), whereas the numbers of IFN-γ-secreting lymphocytes exceeded that of IL-4-secreting cells among most CP subjects.

The increased ratio of IL-4 to IFN-γ-secreting cells was restricted to filarial antigens. The Fo of PPD-driven IFN-γ-secreting lymphocytes was equivalent for MF and CP subjects (GM = 1:5,710; range, 1:2,370–12,050; and GM = 1:3,944; range, 1:917–47,170, respectively; P = 0.2) and PPD induced rare IL-4-secreting lymphocytes with a Fo that was also not significantly different between the two groups (CP, GM = 1:9 × 10^6; and MF, GM = 1:3.3 × 10^6; P = 0.31).

IL-10 and TGF-β mRNA levels in MF vs. CP individuals. In addition to IL-4, IL-10 has also been associated with a T helper type 2 (Th2) pattern of cytokine production in murine models of helminth infection and correlates with downregulation of Th1 responses (16, 26–28). Although a more ubiquitously produced cytokine than IL-10, TGF-β also suppresses lymphocyte proliferation (17). To examine whether MF subjects produced more IL-10 and/or TGF-β compared with CP individuals, mRNA levels were measured in filarial Ag-stimulated PBMC. IL-10 mRNA transcripts were present in five of five MF subjects studied, whereas comparatively little IL-10 production occurred in CP individuals (Fig. 2). This difference was statistically significant based on a comparison of the ratio of IL-10 to HPRT density units, (CP, mean = 0.11±.01 vs. MF, mean = 0.57±0.31; $P < 0.01$). Ag-induced TGF-β mRNA was present in four of five MF individuals and in two of the four CP individuals examined (data not shown).

IL-10 and TGF-β suppress Ag-driven T cell proliferation responses in MF and not CP subjects. Since IL-10 production was preferentially elevated among MF subjects compared with CP individuals and a principal action of this cytokine is to inhibit antigen-driven lymphocyte proliferation (27–29), the role of IL-10 in mediating the Ag-specific "defect" in proliferative response seen in MF was examined in the presence of neutralizing Ab to IL-10. Anti-IL-10 significantly augmented by 220–1,300% BmA-driven T cell proliferation in 7 of 10 MF patients, whereas it had no effect on T cell proliferation in the 11 CP subjects studied ($P < 0.01$; Fig. 3, *left*). Note the overall

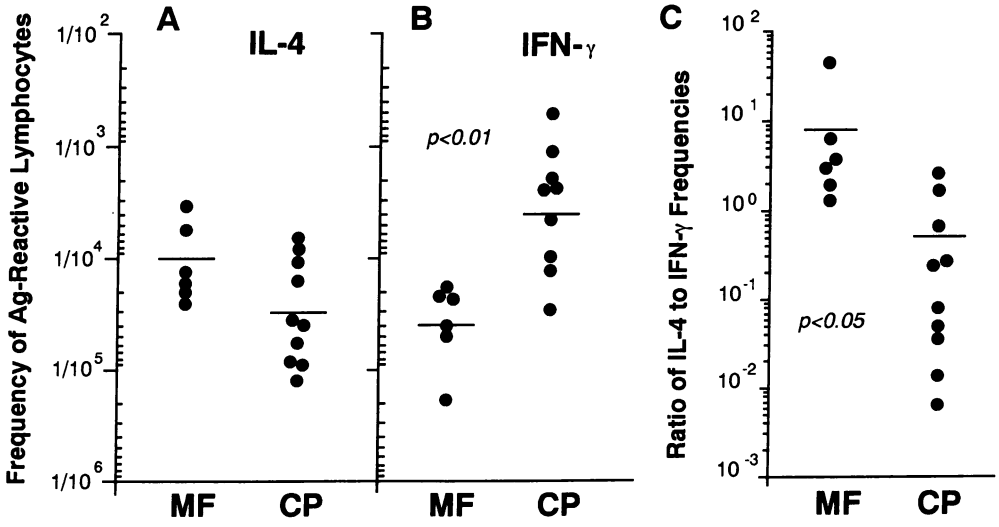

Figure 1. Frequency of Ag-reactive lymphocytes in PBMC secreting IL-4 (*A*) or IFN-γ (*B*), or the ratio of the frequencies (*C*) from patients with CP and MF. Each point represents one individual and the horizontal lines indicate the geometric means.

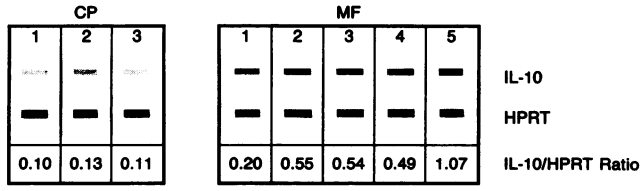

Figure 2. Levels of IL-10 and HPRT mRNA in parasite antigen-driven PBMC from individuals with CP or MF. The ratio of denistometric units for IL-10 and HPRT mRNA is shown below each individuals' autoradiographs.

diminished T cell proliferative response in MF (vis-a-vis CP subjects) as evidenced by the different scales on the *y*-axis in Fig. 3. Although T cell proliferation was augmented in most MF subjects in the presence of neutralizing IL-10 antibody, even the enhanced proliferation among MF subjects generally remained below that of the CP patients.

TGF-β mRNA levels were also elevated in two MF subjects and may also contribute to the modulation of lymphocyte proliferation responses (17). In parallel experiments, neutralizing anti-TGF-β Ab added to cell cultures also significantly augmented, although to a lesser degree, Ag-driven T cell proliferation in PBMC from 5 of 10 individuals with MF, but caused no enhanced response by T cells of CP individuals ($P < 0.05$) (Fig. 1).

The effect of neutralizing anti-IL-2 and anti-IFN-γ antibodies on T cell proliferation in both groups was studied in parallel to that of anti-IL-10 and anti-TGF-β. Neutralizing anti-IL-2 Abs significantly inhibited Ag-driven T cell proliferation in 3 of 10 MF and 8 of 11 CP subjects. Neutralizing anti-IFN-γ Ab had no consistent effect on T lymphocyte proliferation in either group (data not shown).

Discussion

Impaired lymphocyte proliferation responses to filarial antigens have been consistently observed in individuals with circulating MF compared with amicrofilaremic subjects (4, 5, 11, 12). The recent understanding that lymphocytes produce a broad array of cytokines that may influence the pattern of host responses suggested further study of cytokine production in filarial infections. The present study demonstrates that MF individuals possess filarial Ag-reactive lymphocytes that respond fully to filarial antigens; their response, however, was primarily in IL-4 production (and not IFN-γ secretion). This contrasted with the more immunologically responsive CP individuals, who had a greater number of Ag-driven IFN-γ-secreting cells

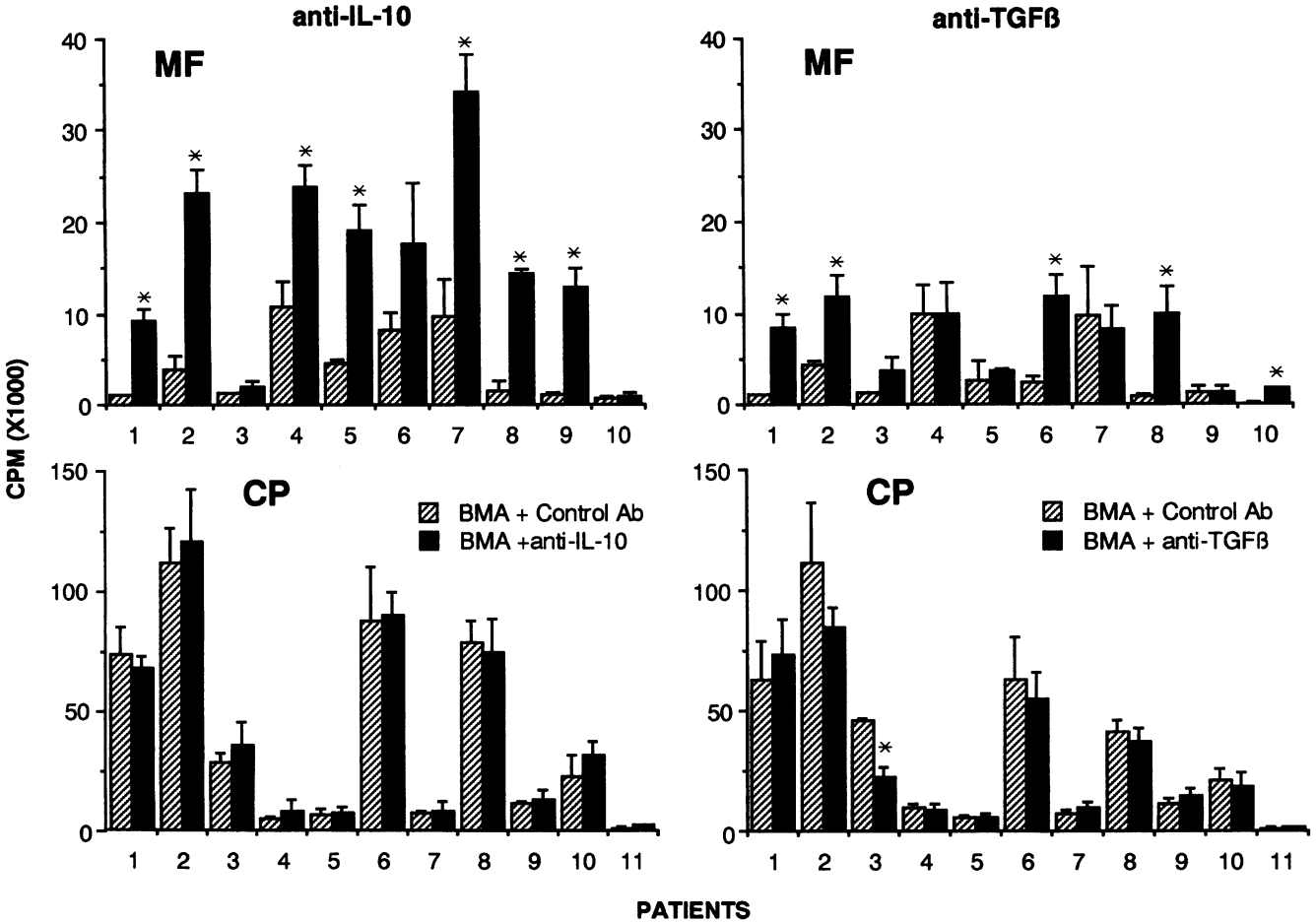

Figure 3. Effect of neutralizing anti-IL-10 and anti-TGF-β Abs on parasite Ag-driven T cell proliferation. Each paired set of bars represents an individual's mean (±SD) net parasite Ag-driven proliferation (cpm) of triplicate cultures in the presence of the appropriate control Abs or with anti-IL-10 Ab (*left*) or anti-TGF-β Ab (*right*). Asterisks represent values that are significantly different from controls ($P < 0.05$).

relative to IL-4. This pattern of cytokine production was restricted to filarial antigens as the Fo of IL-4- and IFN-γ-secreting cells to the nonparasite Ag, PPD, were equivalent between the two groups. The predominant IL-4 response to filarial Ags and elevated serum levels of polyclonal IgE and filarial-specific IgG4 suggests a Th2-like pattern of cytokine production. In contrast, the increased numbers of filarial Ag-specific IFN-γ-secreting cells, strong lymphocyte proliferation responses, and previous observations of enhanced IL-2 production (relative to MF subjects in response to filarial Ags [14]) demonstrated a Th1-like pattern of cytokine synthesis among symptomatic, amicrofilaremic individuals (CP). Thus, the impaired lymphocyte proliferation among MF subjects results not from a lack of Ag-reactive lymphocytes, but from a pattern of T cell activation that generates cytokines (increased IL-4 and diminished IFN-γ), which promotes humoral responses and not lymphocyte proliferation.

Although less potent than IL-2, IL-4 also induces T cell proliferation (30), and the almost complete absence of T cell blastogenesis in some MF subjects observed in the present and previous studies (4, 5, 11) suggested downmodulation of the Th1 response. To address this question two cytokines that suppress lymphocyte proliferation were examined: IL-10 and TGF-β (17, 27–29). Ag-driven IL-10 mRNA levels were clearly elevated in MF compared with CP individuals. Although Ag-induced TGF-β mRNA levels were elevated in 80% of MF vs. 50% of CP subjects, the number of individuals studied was too small to draw any conclusions about significant differences between the two groups. More importantly, neutralizing Abs to IL-10 and TGF-β significantly enhanced Ag-specific lymphocyte proliferation among MF and not CP subjects, indicating that these cytokines contribute to a component of active downmodulation. Whether IL-4 also contributes to this cross-regulation is currently being investigated.

The principal subpopulation of PBMC responsible for increased IL-10 production by MF subjects remains to be defined, however, recent studies in normal human PBMC suggest monocytes are a major source of IL-10 (27). Indeed, monocyte (or B cell)-derived IL-10 could account for the findings in a previous study in brugian filariasis demonstrating that depletion of adherent cells from PBMC can partially reverse the impaired T cell proliferation in MF subjects (8). The mechanism of this IL-10 suppression of lymphocyte proliferation likely occurs by inhibiting APC expression of MHC II (28) or certain molecules such as B7/BB1 necessary for T cell activation (31).

Recently, in a study of a clinically similar group of MF subjects (S. Mahanty, unpublished observations), neutralizing Ab to IL-10 failed to augment Ag-specific IL-4 or IFN-γ production, although in this set of patients lymphocyte proliferation to parasite Ag tended to be roughly twofold greater than that observed in the present study. Clearly, the precise role that IL-10 plays in suppressing lymphocyte responses in MF subjects remains to be fully defined. Nevertheless, modulation of lymphocyte responses in patients with MF are likely to be multifactorial with a predominant Th2 response to filarial Ags contributing the major component. Active downmodulation by cross-regulatory cytokines such as IL-10 and TGF-β may contribute both to an Ag-specific component (T cell–derived IL-10) and an Ag-nonspecific component (APC-derived IL-10 and TGF-β).

The relative expression of filarial antigen-driven IFN-γ and IL-4 secretion by individual lymphocytes appears to mirror the clinical spectrum of response in lymphatic filariasis. The downregulation of IFN-γ production and lymphocyte proliferation in MF subjects may diminish induction of cellular effectors that contribute to the pathology of the disease and facilitate removal of the microfilariae. In addition, enhanced IL-4 synthesis generates strong humoral responses, particularly polyclonal IgE, that may facilitate persistence of infection through the production of irrelevant (not parasite Ag-specific) IgE that saturates high affinity IgE receptors expressed on mast cells and renders them unable to be specifically cross-linked by parasite antigen.

The presence of strong Th2 responses to helminth infections has been well established in murine models of *Schistosoma mansoni* (32), *Nippostrongylus brasilensis* (33), *Heligmosomoides polygyrus* (34), and *Strongyloides venezuelensis* (35). The relative balance of cytokines associated with Th1 and Th2 subsets of $CD4^+$ cells may determine the outcome of the host response to infections with Leishmania (36), Trichinella (37), Schistosome (38), and Nippostrongylus species (33). In murine models of schistosomiasis, the induction of a Th2 response with egg deposition and associated suppression of a Th1 response correlated with increased IL-10 production (26). Similar to observations in the present study, addition of neutralizing anti-IL-10 Ab to antigen-driven lymphocyte cultures from schistosome-infected or (26) filarial-infected mice (39) enhanced T cell proliferation and Th1 cytokine production. These studies suggest an analogous immunomodulatory role of IL-10 in murine models of helminth infections.

Profiles of cytokine responses in relation to the spectrum of disease have also been examined in human schistosomiasis. Humans with chronic schistosome infections are often asymptomatic, have diminished granuloma size (40), and manifest depressed antigen-specific T cell proliferation, IFN-γ production, and IL-2 production along with augmented mitogen-driven IL-4 synthesis in culture supernatants of PBMC (41, 42). After specific chemotherapy for schistosomiasis, parasite Ag-driven T cell proliferation (43), along with IFN-γ and IL-2 production by PBMC, increases significantly (41), thereby indicating a depressed Th1-like response during chronic infections.

The preferential induction of IL-4 relative to IFN-γ production (Th2-like response) in MF subjects may result from in utero (or perinatal) exposure to parasite antigens or to antiidiotype antibodies that render lymphocytes "unresponsive" to parasite antigens (as determined by lymphocyte proliferation). Indirect evidence for prenatal sensitization has come from findings that antifilarial antibodies of isotypes that do not cross the placenta (e.g., IgE and IgM) can be identified in the cord blood of babies born to infected mothers (44) or in the cord sera of babies born in a region endemic for *W. bancrofti* (45). This neonatally induced tolerance may be restricted to the Th1 subset whereas Th2 responses may escape the induction of tolerance, a possibility supported by recent studies in which alloantigens given to neonatal mice resulted in donor-specific expansion of Th2 cells (46, 47). Thus, lymphocytes obtained from infant cord blood or young children born from microfilaremic mothers may preferentially produce IL-4 compared with IFN-γ or IL-2 in response to parasite antigen stimulation, in contrast to the offspring of amicrofilaremic mothers.

Alternatively, the preferential induction of a Th2-like response in MF subjects may result from peripheral tolerance. This concept implies that a state of proliferative unresponsiveness is caused by an absence of a costimulatory signals from the APC. Therefore, acquisition of infection in childhood may allow establishment the microfilaremic state, which, in turn, leads to a high intravascular antigen load that saturates resting B cells (or other APC [48]) or fails to induce costimulatory ligands that could then lead to the preferential induction of Th2 responses (49).

In the present study, we have demonstrated that MF subjects respond to filarial Ags and produce cytokines (e.g., IL-4, IL-10, and TGF-β) that have an antiinflammatory effect. Furthermore, these data suggest that cytokine cross-regulation can contribute to the impaired T cell proliferation observed among MF subjects and may be diminished further by a relative decrease in the Fo IFN-γ-secreting lymphocytes (and by inference IL-2-secreting cells) compared with IL-4-producing cells. Most importantly, these data indicate that our definition of tolerance should not be limited to the absence of Ag-reactive proliferation of T cells but should be broadened to include the notion that states of "specific immune unresponsiveness" may be a reflection of an altered balance of immune reactivity.

Acknowledgments

We thank A. Maya, S. Usha, and S. Kumari for their technical assistance throughout all aspects of this project. In addition, we thank Pat Amato for help in preparation of the manuscript and Dr. James Kazura for his thoughtful discussions and critical review of the paper.

The DNAX Research Institute is entirely supported by the Schering-Plough Corporation.

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