Cytokine Control of Parasite-Specific Anergy in Human Urinary Schistosomiasis

IL-10 Modulates Lymphocyte Reactivity[1]

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Humans chronically infected with schistosomiasis usually have impaired parasite Ag-specific lymphocyte proliferation and IFN-γ production that may facilitate persistence of the parasite while producing little clinical disease. The mechanisms that contribute to the immunologic hyporesponsiveness in these patients remain undefined. IL-10 has been shown to exert an inhibitory effect on cell-mediated immunity. To determine whether endogenous IL-10 has a role in regulating parasite-specific anergy in schistosomiasis, neutralizing anti-IL-10 added to PBMC from *Schistosoma haematobium* patients' enhanced adult worm (SWAP)- or egg Ag (SEA)-driven lymphocyte proliferation and/or IFN-γ production by 2- to >100-fold in 32 of 38 subjects. In contrast, anti-IL-10 failed to significantly augment the mycobacterial Ag, purified protein derivative (PPD)-driven lymphocyte proliferation, or IFN-γ production in 9 or 10 of 14 individuals, respectively. SWAP or SEA triggered IL-10 release from PBMC of both patients and healthy individuals; however, $CD4^+$ cells were a significant source of IL-10 only in infected subjects. PPD relative to SWAP induced fivefold less IL-10 release by $CD4^+$ cells ($p < 0.01$). A possible mechanism whereby IL-10 suppressed Ag-specific T cell responses was demonstrated by the ability of SWAP and not PPD to suppress B7 expression on PBMC. Anti-IL-10 completely inhibited the parasite Ag-induced down-regulation of B7 expression. These studies indicate that IL-10 contributes to parasite Ag-induced T cell hyporesponsiveness observed in patients with chronic schistosomiasis hematobia. *The Journal of Immunology,* **1996, 156: 4715–4721.**

Schistosomiasis generates a range of host immune responses that may contribute to the pathogenesis of this disease (1). Eggs released by adult worms become trapped in host tissues, forming a granulomatous inflammation (2). In some individuals this leads to severe fibrosis and liver cirrhosis. However, most chronically infected individuals generate small granulomas with little disease (3). This may reflect suppressed or impaired host inflammatory responses to the parasite.

The spectrum of pathologic responses correlates with levels of Ag-specific cell-mediated responses (4–7). PBL (or spleen cells) from asymptomatic *Schistosoma mansoni*-infected individuals proliferate poorly in response to parasite Ags compared with 1) acutely infected individuals, 2) subjects after curative chemotherapy, or 3) patients with clinically apparent disease. The mechanism of immune modulation in these persons is poorly understood. Several hypotheses have been proposed that include generation of certain anti-Id Abs that suppress T cell reactivity (7–9); monocyte-derived molecules (10–12); or cross-regulatory cytokines produced by Th2 subsets (13). A key cytokine in this network is IL-10, which has been shown to inhibit lymphocyte proliferation and IFN-γ production in murine models of schistosomiasis (14, 15). IL-10 also modulates T cell responses in human infections. Parasite Ag-induced IL-10 suppresses T cell proliferation in human lymphatic filariasis (16), leprosy (17), and visceral leishmaniasis (1, 18, 19). IL-10 inhibits T cell activation by several mechanisms, such as suppression of monokine production and down-regulation of MHC II and B7 expression on APCs (20, 21). Decreased expression of B7 can impair the interaction with its ligand, CD28, and block costimulation of egg Ag-specific T cells that are critical for generation of the granulomatous response.

Macrophages represent a major source of IL-10 during infection; however, B cells, mast cells, and Th2 cells also produce IL-10. Indeed, other cell types, particularly egg Ag-specific Th cells, may be essential in modulating the granulomatous response, since immunosuppression in human schistosomiasis is Ag specific. In contrast, purified protein derivative (PPD)[3]-driven T cell responses remain intact in subjects with chronic helminth infections (16), even though heat-killed mycobacteria or PPD stimulate significant IL-10 release by monocytes (22, 23). Thus, we postulate that IL-10 released by Ag-specific $CD4^+$ contributes to the impaired T cell response observed in chronically infected subjects. The present study examines the role of endogenous IL-10 in maintaining the impaired T cell proliferation and IFN-γ production by PBMC in chronically *Schistosoma haematobium*-infected subjects.

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Received for publication December 26, 1995. Accepted for publication March 14, 1996.

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[1] This work was supported by Schistosomiasis Research Project Grant 06 03 61 (U.S. Agency for International Development and the Egyptian Government) and Research Career Development Award (to C.L.K., National Institutes of Health Grant 01202-01).

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[3] Abbreviations used in this paper: PPD, purified protein derivative; SWAP, adult *S. haematobium* worm antigen; SEA, *S. haematobium* egg antigen; SI, stimulation index; HPRT, hypoxanthine-guanine phosphoribosyl transferase.

0022-1767/96/$02.00

Materials and Methods

Study population

Thirty-eight volunteers were recruited from villages located near Assiut, in the Assiut Governorate, Upper Egypt. This area is endemic for *S. haematobium* with rare foci of *S. mansoni* transmission (24). Study subjects ranged from 6 to 30 years of age (median age = 14; $n = 38$), and all but two subjects were male. Selection was based on the availability of patients for study. Geometric mean egg count was 15/10 ml urine (range = 1 to 261) determined by at least two separate mid-day urine filtrations through a nucleopore filter. Four to six percent of subjects had never been treated, and the remaining individuals had received treatment more than 1 yr before study. Dysuria was the most common symptom (74% of subjects). Urinary tract abnormalities (as determined by ultrasonography and attributable to *S. haematobium* infections based on their disappearance after chemotherapy) occurred in 28% of study subjects. Other detectable helminth infections (e.g., hookworm, Ascaris, or Trichuris), based on a single stool examination, were present in 22% of subjects. Uninfected subjects were obtained from the city of Assiut itself where schistosome transmission does not occur.

Ags, Abs, and reagents

Adult *S. haematobium* worm Ag (SWAP) and egg Ag (SEA) were prepared as saline extracts as previously described (24). Endotoxin levels in these preparations were 32 μg/mg SWAP, 12 μg/mg SEA and 16 μg/mg PPD (Lederle Labs, American Cyanamide, Wayne, PA). When these Ags were diluted to concentrations used in the experiments, endotoxin levels are 5- to 50-fold less than that required for LPS stimulation of IL-10 from human monocytes (25). Neutralizing anti-IL-10 (mAb JES3-19F1, or rat IgG2a mAb GL117, as an isotype-matched control, PharMingen, San Diego, CA) was used at a concentration of 10 μg/ml. B7 expression was measured by indirect immunofluorescence using a biotinylated CTLA4-IgG1 fusion protein (kindly provided by Dr. Gary Gray at Repligen Corp., Cambridge, MA). The expressed human IgG1 heavy chain alone was biotinylated and used as a control. Stained cells were fixed with 4% paraformaldehyde, stored at 4°C, and examined by flow cytometry within 2 wk.

Culture conditions for in vitro cytokine production

PBMC were purified from heparinized venous blood by sedimentation on a Ficoll-diatrizoate gradient. Cell cultures for the measurement of cytokine production were performed in RPMI 1640 containing 10% FCS, gentamicin (80 μg/ml), and 25 mM HEPES (C-RPMI). PBMCs were cultured at 2×10^6 cells/ml in C-RPMI in a total volume of 1 ml. To duplicate cultures were added either medium alone, SEA (at 5 and 20 μg/ml), SWAP (at 20 and 50 μg/ml), the mycobacterial Ag, PPD at 5 μg/ml or PMA (50 pg/ml) with ionomycin (1 μg/ml; Calbiochem, La Jolla, CA). Cells were incubated at 37°C in 5% CO_2 and supernatants collected at 24, 48, and 72 h and immediately frozen at −70C for subsequent determination of cytokine production. For lymphocyte proliferation assays, PBMC were adjusted to a concentration of 1×10^6 cells/ml in RPMI 1640 containing 10% pooled human serum, gentamicin (80 μg/ml), and 25 mM HEPES and cultured at a volume of 0.2 ml in 96-well microtiter plates. Cells were stimulated in triplicate cultures with either SEA, SWAP, PPD (10 μg/ml), or pokeweed mitogen (1:200 dilution, Life Technologies, Detroit, MI) and pulsed with 1 μCi of [^{3}H]thymidine after 6 days and harvested 12 h later. Lymphocyte proliferation is expressed as stimulation indices (SI = cpm of stimulated cultures divided by the cpm of unstimulated cultures). Optimal SWAP- and SEA-driven IFN-γ production occurred at 20 μg/ml. At this concentration, parasite Ags failed to stimulate cytokine production by PBMC from normal subjects (data not shown).

Cytokine production by lymphocyte subsets

To assess cytokine production by lymphocyte subpopulations, PBMC were initially stimulated in the presence or absence of Ag or mitogen for 24 h prior to immune magnetic positive selection. The stimulated lymphocytes were then washed once in cold PBS, and resuspended in cold HBSS lacking Ca^{2+} and Mg^{2+} (Life Technologies) with 1% BSA. Cell selection was performed using either magnetic beads directly conjugated with CD4 or CD19 (Dynal Corp., Lake Success, NY) or magnetic beads coated with goat anti-mouse Ig (Dynal Corp.) that were conjugated to ammonium sulfate-precipitated ascites fluid containing either CD16 or OKM1 for monocyte selection. Immunomagnetic sorting was performed according to manufacturers instructions. $CD4^+$ cells were selected first, followed by monocyte selection. Cell enrichment yielded >95% $CD4^+$ cells and <2% monocytes. Enriched cells were then cultured in 200 μl of culture medium for an additional 24 h. The precise number of cells after positive selection was not determined in all study subjects. Selected cells were counted in three infected and four uninfected individuals with approximate cell concentrations of 2×10^6 $CD4^+$/ml and 2×10^5 monocytes/ml.

Analysis of cytokine mRNA

Total RNA was extracted from 1×10^6 CD4 cells using RNAzol B (Teletest, Friendswood, TX) and stored at −70°C until use. Total RNA concentration was measured by spectrophotometry.

Cytokine transcript levels were measured using a semiquantitative reverse transcriptase PCR technique previously described (26). Briefly, 1 μg of total RNA was reverse transcribed using Moloney murine leukemia virus reverse transcriptase (Life Technologies, Gaithersburg, MD) 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 Corp., Madison, WI). PCR products were separated on a 1% agarose gel and transferred to a Hybond N^+ membrane (Amersham International, Amersham, U.K.) using standard blotting techniques. Southern transfers were subsequently probed with internal cytokine-specific oligonucleotides and visualized using the enhanced chemiluminescence (ECL) detection system (Amersham International, Arlington Heights, IL). Autoradiographs were scanned with a SciScan 2000 (United States Biochemical, Cleveland, OH).

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. 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-guanine phosphoribosyl transferase (HPRT) was made.

The primers and probes for IL-10 and HPRT were prepared on a DNA synthesizer (Synthetic Genetics, San Diego, CA) and the sequences for sense, antisense primers, and probes, respectively, were as follows: IL-10 5′ γCTG AGA ACC AAG ACC CAG ACA TCA A, IL-10 3′ CAA TAA GGT TTC TCA AGG GGC TGG GTC, and IL-10 probe GCC ATG AGT GAG TTT GAC ATC; HPRT 5′ CGA GAT GTG ATG AAG GAG ATG G, HPRT 3′ GGA TTA TAC TGC CTG ACC AAG G, and HPRT probe GCT GAC CTG CTG GAT TAC AT.

IFN-γ and IL-10 assays

Cytokine levels in cell supernatants were measured by ELISA and expressed in picograms per milliliter by interpolation from standard curves based on recombinant lymphokines using Abs and methods previously described (27).

Statistical analyses

Data are expressed as mean ± SE unless otherwise stated. Experimental conditions were compared using Student's t test on log-transformed data. Tests of significance for regression analysis were also performed by t test of log-transformed data. Statistical significance was considered at $p < 0.05$.

Results

Lymphocyte proliferation and IFN-γ production

Since stage-specific immunoregulatory response may differ, both SWAP- and SEA-specific proliferation and cytokine production were examined. Lymphocyte proliferative responses to both SWAP (mean SI = 7.3) and SEA (mean SI = 6.3) were significantly diminished compared with PPD (mean SI = 25.2) (Fig. 1; $p < 0.01$). Many subjects failed to respond to parasite Ags; 18 of 38 subjects failed to proliferate to SWAP and 25 of 38 did not respond to SEA. The criteria for absence of significant lymphocyte proliferation corresponded to the mean + 2 SD by PBMC from healthy subjects in response to SWAP (SI of 3.4) or SEA (SI of 3.0). All study subjects generated strong proliferation response to PHA (Fig. 1).

Both SWAP or SEA stimulated little IFN-γ production (SWAP, geometric mean = 17.8 pg/ml with 17 of 35 subjects producing no detectable IFN-γ; SEA, geometric mean = 14.1 pg/ml with 19 of 35 subjects generating undetectable IFN-γ) (Fig. 1). PPD induced more than 10-fold greater IFN-γ release (geometric mean = 183.1 pg/ml; $p < 0.001$) than parasite Ags. PBMC from all patients generated at least 400 pg/ml of IFN-γ production in response to the mitogens PMA + ionomycin (Fig. 1).

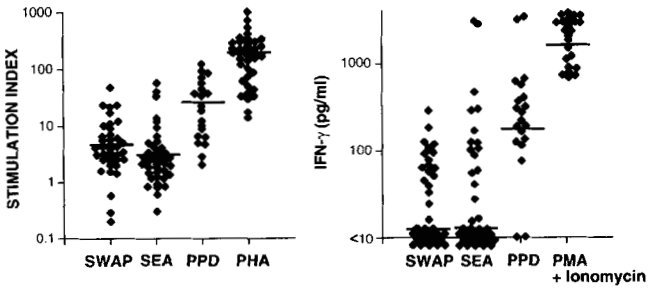

FIGURE 1. Lymphocyte proliferation and IFN-γ production by PBMC from the study population. Each point indicates a separate individual. Lymphocyte proliferation is expressed as a SI (cpm of stimulated cultures/cpm of unstimulated cultures). IFN-γ production represents net cytokine release (Ag- or mitogen-stimulated IFN-γ in culture supernatants minus IFN-γ release in the absence of Ag). Horizontal bars represent mean SI values or geometric mean IFN-γ levels.

IL-10 suppresses lymphocyte proliferation and IFN-γ production

Since previous studies demonstrate endogenously produced IL-10 inhibits lymphocyte proliferation and IFN-γ production by T cells, we examined the hypothesis that this cytokine contributes to the impaired schistosome Ag-specific T cell responses (28). To test this hypothesis, neutralizing anti-IL-10 was added to PBMC cultures. Blocking endogenously produced IL-10 significantly enhanced lymphocyte proliferation in 32 of 38 and 27 of 38 in SWAP- and SEA-driven cultures, respectively (Fig. 2*A*). Lymphocyte proliferation was enhanced as much as 100-fold. The mean percentage increases were equivalent between SWAP (260%) and SEA (250%). Neutralizing anti-IL-10 added to cultures containing PPD failed to significantly enhance lymphocyte proliferation in 9 of 12 subjects (Fig. 2*A*). The percent increase in SWAP-stimulated lymphocyte proliferation in the presence of neutralizing anti-IL-10 correlated with SWAP-driven IL-10 release in parallel PBMC cultures ($r = 0.9$, $p < 0.001$; Fig. 2*B*). A similar relationship was also observed for SEA-stimulated cultures ($r = 0.6$, $p = 0.04$, data not shown). In contrast, the percent increase in PPD-stimulated lymphocyte proliferation in the presence of neutralizing anti-IL-10 failed to correlate with PPD-driven IL-10 release.

A parallel set of experiments also examined the effects of endogenous IL-10 release on IFN-γ production. Addition of neutralizing anti-IL-10 to lymphocyte cultures significantly enhanced IFN-γ release in response to SWAP (22 of 28 patients, 79%) and SEA (19 of 28 patients, 68%) (Fig. 3). As a group, the mean increase in IFN-γ production in the presence of neutralizing anti-IL-10 was 12-fold for SWAP-stimulated cultures (Fig. 3; $p < 0.001$, paired t test). A similar increase was also observed for SEA-driven cultures (7-fold increase, $p < 0.001$). IFN-γ production in cultures with medium alone significantly increased in the presence of neutralizing IL-10 in 6 of 28 subjects, but as a group, the increase in IFN-γ was not significantly different compared with cultures containing the isotype control Ab. In contrast, PPD-driven IFN-γ production was enhanced in 7 of 17 patients (41% and an overall 1.7-fold increase; Fig. 3) in the presence of neutralizing anti-IL-10. Neither SWAP or SEA-induced IL-10 release had any significant effect on IL-4 or IL-5 production (data not shown).

IL-10 production

Parasite Ag-driven IL-10 production by PBMC culture was initially examined at 24 and 72 h. Parasite Ags induced 50 to 70% more IL-10 release at 72 h compared with 24 h cultures (data not shown); thus IL-10 release by PBMC is shown for 72-h cultures (Fig. 4 and Table I). Figure 4*A* summarizes Ag-driven IL-10 release for 27 individuals. SWAP induced significant IL-10 release in 24 of 27 subjects with an overall fourfold increase in IL-10 release compared with unstimulated cultures (Fig. 4, $p < 0.01$). In addition, SEA-induced significant IL-10 release by PBMC in 21 of 27 individuals. PPD also stimulated significant IL-10 release in 17 of 21 subjects, although the overall amount of IL-10 induction by SEA or PPD was less than that observed for SWAP (Fig. 4). IL-10 production varied considerably among individuals. To illustrate

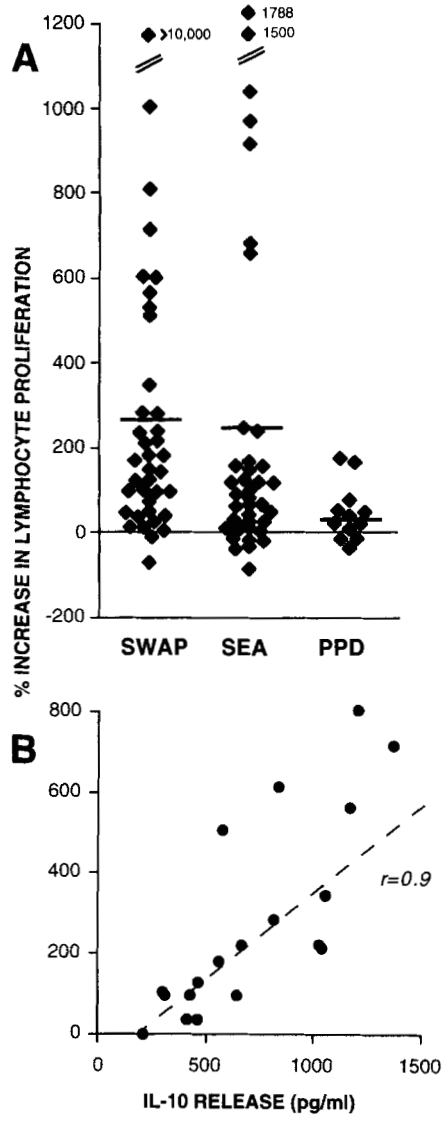

FIGURE 2. Schistosome-induced IL-10 release inhibits lymphocyte proliferation. PBMC were cultured with SWAP, SEA, or PPD and neutralizing anti-IL-10 or isotype-matched control Abs. Six days after initial culture, cells were pulsed with [^{3}H]thymidine and harvested 12 h later. In *A*, values are reported as the percentage increase in cpm: ([cpm in the presence of anti-IL-10 − cpm in the presence of control mAb]/[cpm in the presence of control mAb] × 100). Each point represents a single individual. Mean augmentation of proliferative responses in the presence of anti-IL-10 was significantly greater than in cultures with the isotype-matched control Abs for both SWAP and SEA ($p < 0.01$), but not PPD ($p = 0.3$). *B* shows the correlation between the percentage increase in proliferation responses in the presence neutralizing anti-IL-10 as described in *A* and SWAP-induced IL-10 release by PBMC in a subset of individuals ($r = 0.9$, $p < 0.001$).

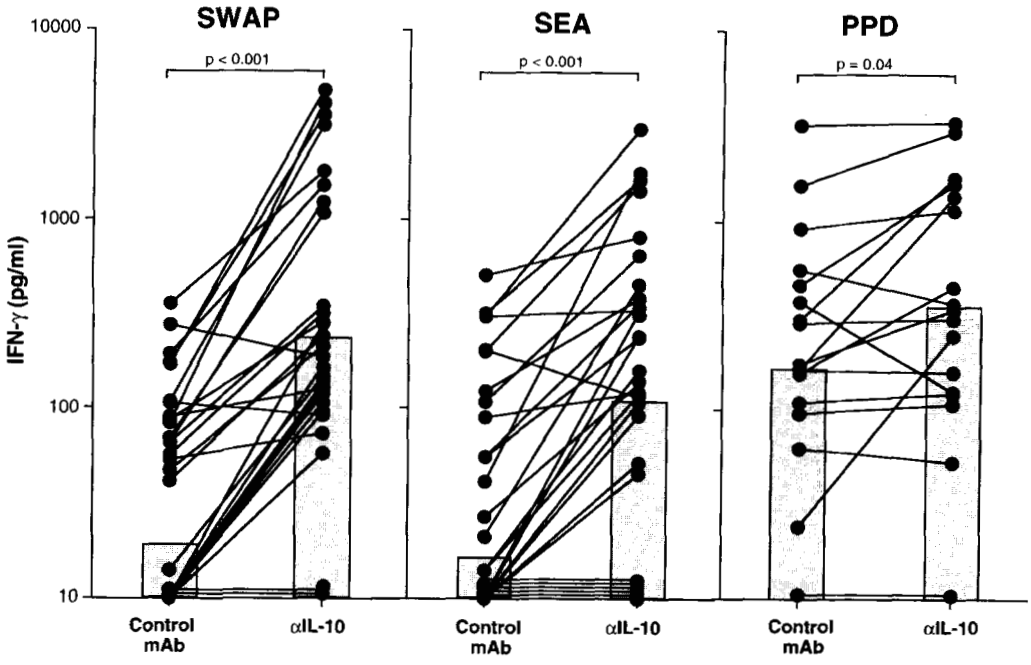

FIGURE 3. Schistosome-induced IL-10 release suppresses IFN-γ production. PBMC were cultured for 72 h with schistosome Ags or PPD and neutralizing anti-IL-10 or isotype-matched control Abs. Each pair of circles connected by a line represents one individual. Panels represent the effect of neutralizing anti-IL-10 on net IFN-γ production in the presence of SWAP, SEA, and PPD, respectively. Statistical significance was determined by a paired *t* test of log-transformed data. Bars indicate geometric means.

Table I. *Ag-induced IL-10 release by PBMC, CD4$^+$, and peripheral monocytes*

Patient No.	PBMC			CD4$^+$			Monocytes		
	Media	SWAP	PPD	Media	SWAP	PPD	Media	SWAP	PPD
158	<20	1495 ± 15[a]	1094 ± 123	0	407[b]	27	118	119[b]	157
168	78 ± 11	1380 ± 23	154 ± 7	185	1595	363	73	126	597
44	490 ± 54	2433 ± 387	1471 ± 97	114	3220	421	237	768	547

[a] Values represent means ± SD of IL-10 release (picograms per milliliter) of duplicate or triplicate cultures cultured for 72 h at 2 × 10^6/ml.
[b] Values are IL-10 release (picograms per milliliter) of positively selected cells after 24-h stimulation with or without Ag and cultured an additional 24 h in single cultures in a 96-well microtiter plate.

the range of responses among individuals, data are shown for 3 of the 28 subjects in which cytokine responses were examined in detail (Table I). For example, constitutive IL-10 ranged from undetectable to 490 pg/ml among individuals. There was no relationship between spontaneous IL-10 release and the magnitude of parasite Ag-driven lymphocyte proliferation or IFN-γ release (data not shown).

Cellular source of IL-10

Since individuals with chronic schistosome infections demonstrate impaired T cell responses to schistosome Ags and not mycobacterial Ags or mitogens, we postulated that parasite Ag-reactive CD4$^+$ T cells contribute to this immunopression by release of IL-10. To examine this hypothesis, PBMC were cultured in the presence or absence of parasite Ags or PPD for 24 h and CD4$^+$ and monocyte subpopulations were then immunomagnetically sorted and cultured an additional 24 h as either CD4$^+$ or monocyte-enriched cells without APCs added. SWAP induced significant IL-10 production by CD4$^+$ T cells relative to unstimulated cultures (Fig. 4 and Table I). Overall, the pattern of parasite Ag-driven IL-10 release by CD4$^+$ cells paralleled that observed for PBMC (Fig. 4). PPD stimulated less IL-10 release by CD4$^+$ cells compared with SWAP or SEA ($p < 0.05$) but induced significantly more IL-10 production by monocytes than either parasite Ag (Fig. 4). Parasite Ags failed to stimulate any IL-10 release by CD4$^+$ from unsensitized North American individuals ($n = 4$, data not shown). In the CD4$^+$-enriched populations, the mitogens PMA + ionomycin induced comparatively high levels of IL-10 (Fig. 4) as well as IL-5 (geometric mean of 898 ± 16 pg/ml) and little IL-1 (<0.1 μg/ml), indicating the cytokine pattern of the selected CD4$^+$ cells is characteristic of T cells and is unlikely to be contaminated by many monocytes.

Since neither CD4$^+$ cells nor monocytes were counted after selection (although in pilot experiments using identical selection procedures a fairly consistent cell number was recovered among individuals (see *Materials and Methods*), a comparison of the relative amounts of IL-10 produced by the two subpopulations could not be made. To address this problem, RNA was extracted from both CD4$^+$ cells and monocytes immediately after selection and the levels of HRPT and IL-10 mRNA were determined by semiquantitative PCR. Individuals in whom HPRT mRNA levels were equivalent between CD4$^+$ and monocyte-enriched preparations were used to compare the relative amounts of IL-10 mRNA (n =

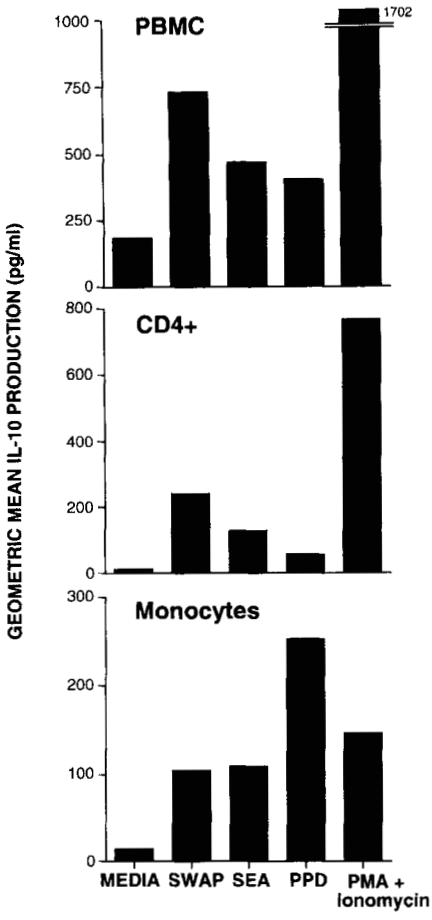

FIGURE 4. IL-10 release by PBMC, $CD4^+$, and monocytes. PBMC (2×10^6/ml) were stimulated for 72 h. $CD4^+$ cells and monocytes were selected from PBMC after 24-h stimulation and cultured an additional 24 h. Each bar represents geometric mean IL-10 release in medium alone ($n = 27$), or in response to SWAP ($n = 27$), SEA ($n = 22$), PPD ($n = 21$), or the mitogens PMA + ionomycin ($n = 17$). SE were less than 10% of means in all cases.

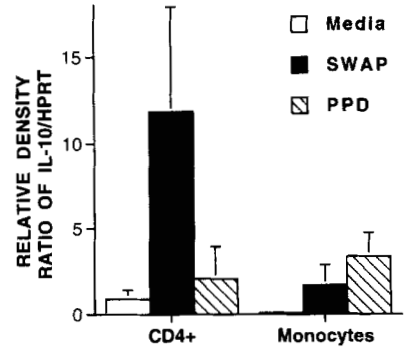

FIGURE 5. Effect of SWAP vs PPD stimulation on IL-10 mRNA levels in $CD4^+$ vs monocytes. $CD4^+$ cells and monocytes were selected from PBMC after 24-h stimulation and the RNA was immediately extracted. HPRT or IL-10 for both monocytes and $CD4^+$ cells were amplified simultaneously and exposed on the same blot. Those individuals with similar HPRT and mRNA levels are shown ($N = 7$). Bars represents the mean values of the ratios of the ODs for IL-10 to HPRT in response to SWAP (shaded bars), PPD (cross-hatched bars), or medium alone (open bars).

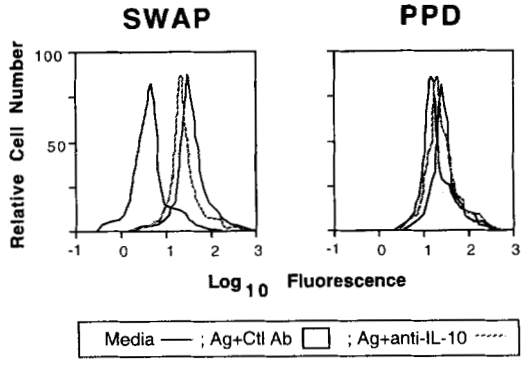

FIGURE 6. Effect of SWAP or PPD stimulation on B7 expression by PBMC from a patient with chronic schistosomiasis hematobia in the presence of neutralizing anti-IL-10 or isotype-matched control mAb. PBMC were stimulated with SWAP or PPD for 24 h prior to cell surface staining. Fc receptors were blocked by prior incubation with pooled human serum, and B7 expression (biotinylated CTLA4-IgG1) was determined by indirect immunofluoroescence and flow cytometry. Control staining was performed with biotinylated control-IgG1. PBMC were gated for large mononuclear cells, and 5000 cells were analyzed per sample. The patient shown is representative of four similarly infected individuals studied.

7). When the levels of SWAP- vs PPD-induced IL-10 mRNA levels were compared (normalized with respect to HPRT), overall, SWAP induced a mean 10-fold increase in IL-10 message compared with unstimulated cells in $CD4^+$ selected cultures (Fig. 5). PPD induced little IL-10 mRNA in $CD4^+$ cells. In contrast, PPD-stimulated significantly greater levels of IL-10 message in the monocyte-enriched fraction compared with SWAP ($p < 0.05$, Fig. 5). The T cell mitogens PMA + ionomycin induced more IL-10 mRNA expression in $CD4^+$ cells compared with monocytes (data not shown).

Endogenous IL-10 inhibits schistosome Ag-induced B7 expression

IL-10 has been shown to suppress T cell function by several mechanisms that includes inhibiting monokine release and expression of the ligands B7-1 and B7-2 on APCs (20, 21). The interaction of B7 with its receptors, CD28 and CTLA4, on T cells provides critical costimulatory signals necessary for T cell activation (29). To determine whether schistosome Ag-induced IL-10 inhibits B7 expression, the levels of B7 were measured in SWAP-driven lymphocyte cultures in the presence of neutralizing anti-IL-10 or an isotype-matched control Ab. SWAP, but not PPD, inhibited B7 expression on PBMC gated for monocytes (Fig. 6). Addition of anti-IL-10 reversed the SWAP-induced inhibition of B7 expression. Culture supernatants from the experiment shown in Figure 6 induced a net production of 1022 pg/ml of IL-10 in response to SWAP compared with 123 pg/ml with PPD stimulation.

Discussion

The various events that regulate immunopathology in schistosomiasis probably relate to diminished or altered schistosome Ag-specific T cell responses, since $CD4^+$ T cells are critical for granuloma formation and fibrosis (30). The mechanisms as to how this might occur has received considerable study recently, but most evidence suggests a network of regulatory molecules and/or cytokines that modulate lymphocyte responsiveness. For example, lymphocytes and/or Abs with anti-idiotypic specificity may regulate schistosome Ag-specific T cell responses (10, 12). Alternatively, it has been shown that partial depletion of adherent cells

augmented lymphocyte responsiveness in chronically infected patients (8). This suggests that a monokine may participate in this immunomodulation. IL-10 is abundantly produced by monocytes, as well as many other cells including T and B cells, and is a potent suppressor of T cell proliferation and IFN-γ production by human lymphocytes in vitro (28). The present study demonstrates that parasite Ag-induced IL-10 release by PBMC from schistosomiasis patients contributes to impaired T cell blastogenesis and IFN-γ production to schistosome Ags. We show that in addition to monocytes, parasite Ag-reactive $CD4^+$ cells also release IL-10 that likely contributes to the reduced Ag-specific T cell proliferation and IFN-γ production observed in schistosomiasis patients.

The focus on sensitized $CD4^+$ cells as a major source of IL-10 in response to parasite Ags is important for generating the Ag-specific nature of the T cell hyporesponsiveness. To examine this question further, we contrasted the pattern of IL-10 production in response to PPD, in which T cell responses were generally intact, to that of schistosome Ags. PPD has been previously shown to stimulate IL-10 by PBMC and monocytes; this was confirmed in our present studies (23). In contrast to parasite Ags, PPD usually generated little or no IL-10 by $CD4^+$ cells. This corresponded with the failure of neutralizing anti-IL-10 to enhance PPD-driven lymphocyte proliferation or IFN-γ production in many study subjects examined. Interestingly, among individuals in whom PPD did induce IL-10 by $CD4^+$ cells, PPD-driven IFN-γ release also tended to augment in the presence of neutralizing anti-IL-10 (data not shown).

Our studies demonstrate that schistosome Ag-induced IL-10 release inhibited B7 expression by peripheral blood cells. Because PBMC were gated on the large mononuclear cell population, the suppressed B7 expression most likely occurred on monocytes. However, activated T cells may also have been detected that are also capable of expressing B7 (31). We cannot exclude the possibility that B7 expression may also be suppressed on B cells. IL-10 has been previously shown to inhibit B7 expression on human APCs (20, 21) as well as MHC class II expression, cytokine production by monocytes, and also has direct inhibitory effects on T cells (25, 28). The suppressive effects of IL-10 on parasite-specific proliferation and IFN-γ production may result from reduced B7 expression, but likely occurs in concert with other inhibitory effects of IL-10 on APC and T cell function. It has recently been recognized that at least two distinct molecules of B7 exist, B7-1 and B7-2 (29). Early studies suggest that T cell costimulation by B7-1 favor Th1 responses, and B7-2 costimulation favors Th2 cytokine production (32). Whether these different B7 isoforms are differentially expressed and inhibited in response to parasite Ags on APCs from infected patients remains to be determined.

IL-10 has been shown to suppress B7 expression and macrophage function in murine schistosomiasis (33). Macrophages isolated from granulomas expressed little B7 compared with splenic APCs. Granulomas added to splenocyte cultures inhibited Ag-specific Th1 responses. Neutralizing anti-IL-10 could restore the ability of granulomas to costimulate.

An immunomodulatory role for IL-10 has also been shown in human filarial infections (16). Hosts often generate little overt reaction to brugian and bancroftian filariasis and are clinically asymptomatic with circulating microfilaria (34). Like human schistosomiasis, these microfilaremic individuals have impaired filarial Ag-specific lymphocyte proliferation and IFN-γ production (35). In these studies it was also observed that addition of neutralizing anti-IL-10 enhanced lymphocyte proliferation responses to filarial Ags (16). The effect of parasite Ag-driven IL-10 on IFN-γ production was not examined. Those studies also demonstrated preferential induction of Th2-associated cytokine response to filarial Ags and postulated that IL-10 was associated with this response. Whether IL-10 originated from Ag-specific Th2 cells was uncertain. The present studies suggest a similar mechanism of immunomodulation in human schistosomiasis and demonstrate that sensitized $CD4^+$ can also produce IL-10.

Whether these observations that endogenous IL-10 production by human PBMC suppresses parasite Ag-driven IFN-γ and T cell proliferation in vitro participate in regulating in vivo granuloma formation and fibrosis is unknown. The role of IL-10 in modulating granuloma formation in murine models of schistosomiasis are controversial (36). In acute infections, an enhanced Th1 response occurs. However, with the beginning of egg deposition, Th2 responses predominate and cross-regulate Th1 cytokine production by increased IL-10 release (37, 38). Yet administration of anti-IL-10 did not affect pulmonary granuloma size in the mouse, but did enhance IFN-γ by draining lymph node cells, indicating that endogenous IL-10 production was partially abrogated (39). Whether the IL-10 participates in the granulomatous response in the liver is unknown. Granulomas in the liver, compared with those in the lung, are very different with the former containing more fibrosis (2). IL-10 may also play an important role in regulating fibrosis. For example, IL-10 inhibits type I collagen synthesis while increasing matrix metalloprotease gene expression in human fibroblasts (40) and suppresses eiconosoid- and fibroblast-stimulating factors produced by monocytes (41).

The mechanisms that regulate granuloma and fibrosis are complex and probably involve multiple pathways. Besides IL-10, other cytokines such as IL-4 and TGF-β may also contribute to immunosuppression in chronic schistosomiasis and other infectious diseases. Alternatively, individual differences in the ability to produce TNF-α may also determine whether severe immunopathology persists (42) (personal observations). The present studies provide a basis for further investigation on how such cytokines modulate cell-mediated responses to parasitic infections in humans.

Acknowledgments

In particular, we appreciate the help and cooperation of the study individuals and their families who willingly cooperated with our study. We would like to thank the Egyptian Ministry of Health for help in providing access to the patients and the facilities to help perform these studies. Dr. Zarzour and his colleagues were invaluable in identifying infected individuals and in facilitating their transport to the Assiut University Hospital. We also thank Drs. James Kazura, Eric Pearlman, and Abram Stavitsky for review of the manuscript.

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