Impairment of Tetanus Toxoid–Specific Th1-like Immune Responses in Humans Infected with Schistosoma mansoni

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After vaccination with tetanus toxoid (TT), TT-specific immune responses in humans infected with Schistosoma mansoni were assessed. Peripheral blood mononuclear cells (PBMC) from vaccinated infected subjects and vaccinated uninfected controls were evaluated for their ability to produce cytokines characteristic of Th1 or Th2 cells (interferon [IFN]-γ or interleukin [IL]-4, respectively) after in vitro restimulation with TT. TT-specific IFN-γ production by PBMC from infected subjects was inversely related to infection intensity and was significantly lower than TT-specific IFN-γ production by control PBMC. PBMC from all of the infected subjects and 3 of the 5 controls analyzed by reverse transcriptase–polymerase chain reaction transcribed the IL-4 gene in response to TT restimulation. Together, these results suggest that S. mansoni–infected persons mount a Th2-like response to the bystander antigen TT, while uninfected persons mount a Th1- or Th0-like response.

In mice and humans infected with Schistosoma mansoni, the predominant parasite-specific T helper (Th) cell response is Th2-like [1, 2]. Th2 cells can develop from naive Th cells after activation through the T cell receptor in an environment containing the cytokine interleukin (IL)-4 [3]. The extent of IL-4 production in infected mice suggests that naive Th cells specific for nonchistosome “bystander” antigens might be induced to differentiate in a Th2 direction as a result of the IL-4–rich environment. Indeed, prior infection of mice with S. mansoni is now known to skew in a Th2 direction immune responses to sperm whale myoglobin [4] and vaccinia virus [5]; normally, these antigens or pathogens induce Th1 responses. Because disease control or progression is often linked to the type of Th subset response induced [6], such immune deviation could affect the outcome of concurrent infections. The ability of the existing immunologic environment in schistosome-infected animals to influence the nature of immune responses to unrelated pathogens has profound implications for the efficacy of vaccines as well. In the current study, we ask whether ongoing human infection with S. mansoni, a parasite known to be a strong promoter of IL-4 production, affects the nature of the immune response induced by vaccination with an unrelated antigen, tetanus toxoid (TT).

Materials and Methods

Study population. The study site chosen (Caatinga do Moura, Brazil) is an area with endemic schistosomiasis in northeastern Brazil where, in spite of the use of molluscicide (begun in 1963) and mass chemotherapy with oxamniquine (begun in 1982), S. mansoni infection remains widespread [7]. Mycobacterium bovis bacille Calmette-Guérin (BCG) vaccination at birth is mandatory in Brazil, precluding the use of this predominantly Th1-promoting antigen [8] for our study. Instead, TT was used as the bystander antigen; TT vaccination is not universal in Brazil. Beginning in October 1992, numerous subjects with the intestinal form of schistosomiasis were screened by ELISA for serum antibodies against TT, and 14 were found to be negative. These persons were vaccinated according to the Brazilian Ministry of Health, using 0.5 mL (10 Lf) of TT (Connaught Laboratories, Willowdale, Canada) intramuscularly three times at 1-month intervals. TT-specific immune responses were evaluated 1–5 months after vaccination. Patients were considered to be heavily infected with >400 eggs/g of feces (epg), moderately infected with 200–400 epg, and lightly infected with <200 epg. Uninfected, previously vaccinated subjects not living in the schistosomiasis-endemic area and strongly seropositive as determined by ELISA for TT-specific antibodies were used as controls. The characteristics of the study population are shown in table 1.

Cell preparation and culture conditions. Peripheral blood mononuclear cells (PBMC) were separated from 30–50 mL of heparinized blood by density gradient centrifugation as described [7]. For in vitro stimulations, cells were resuspended in RPMI 1640 containing 15% normal human serum (AB+, heat-inactivated), 100 U/mL penicillin, 100 μg/mL streptomycin, 2 mM L-glutamine, 30 mM HEPES, and 5 × 10−5 M 2-mercaptoethanol (all from Life Technologies GIBCO BRL, Gaithersburg, MD) at 5 × 106/mL and stimulated with TT (20 Lf/mL final; Wyeth Ayerst Laboratories, Marietta, PA) or with the mitogens phytohemagglutinin (1:10 final dilution) or concanavalin A (5 μg/mL; Pharmacia, Piscataway, NJ); the latter was used for vaccinated uninfected controls. For proliferation assays, cells were resuspended in the above medium...
at 10⁵/mL and stimulated with TT at a final concentration of 2 Lf/mL for 5 days.

Measurement of secreted cytokines and of proliferation. Cytokines in 72-h supernatants from in vitro–stimulated PBMC were measured by two site ELISAs. For the interferon (IFN)-γ assay, monoclonal antibody (MAb) 1598-00 (Genzyme, Cambridge, MA) was used as the capture antibody and a rabbit anti-recombinant human IFN-γ (gift of T. Nutman, NIAID, NIH, Bethesda, MD) was used to detect bound cytokine. To detect IL-4 and IL-10, MAbs 8D48 and biotinylated MP4-25D2 or JES3D7 and biotinylated JES312G8 (Pharmingen, San Diego), respectively, were used as directed by the manufacturer. Recombinant cytokines (gift of S. Reed, Seattle Biomedical Research Institute) were used to generate standard curves. Cell proliferation in response to TT was measured by [³H]thymidine incorporation during the final 6 h of in vitro culture.

Reverse transcriptase–polymerase chain reaction (RT-PCR). After the collection of supernatants at 72 h, cells were lysed in RNAzol (Tel-Test, Friendswood, TX), and RNA was prepared according to the manufacturer’s directions. cDNA was generated from 1 μg of RNA using a first-strand cDNA synthesis kit (Pharmacia). Cytokine-specific RT-PCR was done using oligonucleotide primers and conditions as described [9]. RT-PCR products were separated on 1.5% agarose gels, transferred to Nytran (Schleicher & Schuell, Keene, NH) by Southern blotting, and probed with a portion of the corresponding human gene labeled with fluorescein (plasmids provided by S. Reed). Hybridization was detected by enhanced chemiluminescence (Amersham, Arlington Heights, IL). All cDNA samples were amplified using β-actin–specific primers to ensure that equivalent amounts of cDNA per sample were used for cytokine-specific amplification.

Statistical analysis. For cytokine assays, the majority of supernatants were analyzed in duplicate. Data are expressed as means ± SEs. Comparisons between groups are by Student’s t test. P < .05 is considered significant.

Results

Subjects were selected for the study on the basis of being stool-positive for schistosome eggs but seronegative for TT. After vaccination, 13 of 14 infected subjects seroconverted to become positive in the anti-TT ELISA (not shown), indicating that infected subjects do mount a TT-specific Th response after vaccination. This conclusion was supported by the observation that PBMC from vaccinated infected subjects exhibited a pronounced proliferative response to TT (table 1). The strong TT-specific antibody response demonstrated by the previously vaccinated controls (not shown) was considered sufficient evidence of a Th cell response to vaccination; lymphoproliferative responses to TT were not evaluated in these subjects.

We had hypothesized that the background immunologic status of the schistosomiasis patients would predispose toward development of TT-specific Th2 cells. To investigate this, we restimulated PBMC from vaccinated subjects with TT in vitro and measured IL-4 by ELISA and in some cases analyzed IL-4 transcripts by RT-PCR. TT-induced IL-4 secretion was not detectable by ELISA. However, when RT-PCR was used, IL-4 transcripts were evident in TT-stimulated PBMC from the 5 infected subjects tested and from 3 of 5 uninfected controls (figure 1C and not shown).

In previous studies using schistosome-infected mice, immune responses to bystander antigens were characterized by a lack of the Th1 cytokine IFN-γ [4, 5]. To address this issue, we analyzed IFN-γ production by TT-stimulated PBMC from the vaccinated infected and uninfected subjects. PBMC from uninfected subjects secreted a mean of 21 U/mL IFN-γ when stimulated in vitro with TT. In contrast, PBMC from heavily infected persons failed to produce detectable IFN-γ when restimulated with TT (figure 1A). PBMC from moderately infected subjects produced IFN-γ in response to TT, but the amount produced, 1.7 U/mL, was > 1 log less than that produced by cells from uninfected controls (P = .0031, figure 1A). PBMC from lightly infected subjects secreted an intermediate level (7.6 U/mL) of IFN-γ (P = .04 vs. uninfected controls, figure 1A), suggesting that the degree of IFN-γ down-regulation is related to the intensity of infection with S. mansoni. The relative inability of PBMC from infected persons to produce IFN-γ was TT-specific, since in vitro stimulation with mitogen led to the production of large amounts of IFN-γ (figure 1B).

Discussion

TT-specific Th responses in people from areas not endemic for schistosomiasis have been studied previously [10]. The majority of Th cells cloned from PBMC under conditions that did not predispose towards a particular Th subset were either Th1- or Th0-like, with few (~15%) being Th2-like. This suggests that vaccination with TT in persons not infected with S. mansoni normally stimulates the development of a population of Th cells that can make IFN-γ on restimulation. Consistent with this, we show that PBMC from uninfected, vaccinated controls make relatively large amounts of IFN-γ in response to restimulation with TT, while IFN-γ production by TT-restimulated PBMC from infected, vaccinated subjects was unmeasurable or low. This suggests that people with schistosomiasis have an impaired ability to mount Th1-like responses to
Figure 1. Interferon-γ production by peripheral blood mononuclear cells (PBMC) from tetanus toxoid (TT)-vaccinated S. mansoni infected or uninfected subjects after in vitro restimulation with TT (A) or mitogen (B). Columns represent mean ± SE of duplicate assays on 72-h supernatants from PBMC from 7 heavily infected, 5 moderately infected, and 2 lightly infected subjects and from 4 uninfected controls. BKG, cells cultured for 72 h in medium alone. C, Southern blot of interleukin (IL)-4-specific reverse transcriptase–polymerase chain reaction products from RNA isolated from PBMC from representative sample of heavily infected (n = 5) or uninfected (n = 3) subjects after culture for 72 h with TT or in medium alone (Bkg).

The bystander antigen TT. Moreover, the data imply that the more heavily infected the subject is, the greater is the impairment in IFN-γ production. This IFN-γ deficit does not reflect a systemic inability to produce IFN-γ, as can result from schistosomiasis in some strains of infected mice [1], since mitogen-stimulated PBMC from infected, vaccinated subjects make high levels of IFN-γ. In contrast to a previous report in which 31% of infected, TT-vaccinated Egyptian patients did not seroconvert [11], 93% of patients in our study group did mount strong anti-TT antibody responses after vaccination; this difference may be due to the fact that patients in the Egyptian study group had more severe disease than the Brazilian patients we studied.

We had hypothesized that the Th2 nature of the existing schistosome-specific immune response would provide sufficient environmental IL-4 to skew a primary TT-induced response in a Th2 direction. The data presented here in part support such a possibility since, as assessed by RT-PCR, TT-stimulated PBMC from vaccinated infected persons do make IL-4, even though they make low to unmeasurable IFN-γ. Indeed, the failure to produce IFN-γ was the major difference between infected and uninfected vaccinated subjects, as PBMC from 3 of the 5 controls also transcribed IL-4 in response to TT restimulation. Cells from uninfected vaccinated controls, therefore, were Th0- or Th1-like in their TT-stimulated cytokine secretion patterns, similar to that reported previously for TT-specific Th clones from vaccinated people living in nonendemic areas [10]. Additionally, purified protein derivative–stimulated PBMC from S. mansoni–infected persons vaccinated with BCG before parasite infection have been shown to secrete IFN-γ [7, 12], again suggesting that the impairment in TT-stimulated IFN-γ production observed in the current study may indeed be a result of the underlying schistosome-induced Th2-dominated immunologic environment into which the TT vaccine was introduced. The lack of measurable IL-4 in the supernatants of TT-stimulated PBMC is not surprising in light of a recent report that schistosome antigen–stimulated PBMC from persons with schistosomiasis also failed to make ELISA-detectable IL-4 [2].

At present the reason for the difference in TT-induced IFN-γ production between infected and uninfected subjects is unclear. As discussed above, this could be due to predominant priming for TT-specific Th2 cells in S. mansoni–infected people. An alternative possibility is that vaccination with TT primes for a Th0 response but that some infection-associated response specifically down-regulates IFN-γ production by Th cells; IL-10 could be playing such a role [13]. This cytokine, which in humans is produced by macrophages and both Th1 and Th2 cells, is capable of down-regulating Th cytokine production. The TT-stimulated production of IL-10 by cells from some, though not all, infected subjects tested but none of the vaccinated controls was noted (data not shown).

There are no clearcut reports of increased susceptibility of schistosome-infected persons to concurrent infections that might be expected to be controlled by Th1-like cell-mediated immune responses. Nor are there reports of increased resistance to intestinal helminths, which require Th2-like responses for control [14]. Whether this represents the actual situation or simply the lack of appropriate studies is unclear. We would predict on the basis of our findings that in some cases, heavy infection with S. mansoni resulting in a predominantly Th2-type cytokine environment could have a marked effect on the
outcome of concurrent infection or on the efficacy of vaccines directed against other pathogens.

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References

13. del Prete G, de Carli M, Almerigogna F, Giudizi MG, Biagiotti R, Romagnani S. Human IL-10 is produced by both type 1 helper (Th1) and type 2 helper (Th2) T cell clones and inhibits their antigen-specific proliferation and cytokine production. J Immunol 1993;150:353–60.