

Impairment of Tetanus-Specific Cellular and Humoral Responses following Tetanus Vaccination in Human Lymphatic Filariasis

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To investigate the consequences of the impaired parasite-specific immune response in lymphatic filariasis, the effect of concurrent *Wuchereria bancrofti* infection on the immune response to tetanus toxoid (TT) following tetanus vaccination was studied in 20 asymptomatic microfilaremic (MF) patients, 20 patients with chronic lymphatic obstruction/elephantiasis (chronic pathology [CP]), and 10 endemic normal (EN) control individuals at baseline and at 3 and 6 months after TT vaccination. Peripheral blood mononuclear cell (PBMC) proliferative responses to TT before vaccination were not significantly different between the EN control and CP groups, but the MF group showed significantly lower baseline proliferative responses to TT compared with either the EN or CP group. Six months following vaccination, the change in proliferative response to TT was significantly greater in the EN and CP groups than in the MF group. This difference in proliferative response was reiterated in the gamma interferon (IFN- γ) response in the EN group, in that they increased IFN- γ production by 400% at 6 months, in contrast to that seen in the filaria-infected groups. In contrast to the IFN- γ responses, PBMCs from the MF group produced significantly increased levels of TT-specific IL-10 compared with PBMCs from the EN group. Although there was significantly greater TT-specific immunoglobulin G (IgG) production at baseline between the EN and MF groups, postvaccination IgG (and IgG1 isotype) responses did not differ among the groups, whereas TT-specific IgG2, IgG3, and IgG4 were all increased in the EN group compared with the filaria-infected groups. These studies indicate that concurrent infection with *W. bancrofti* can diminish the immune response to an unrelated antigen by a mechanism that is likely to involve IL-10.

Human lymphatic filariasis, a mosquito-borne parasitic disease, is associated with a variety of clinical manifestations, some of which clearly reflect the nature and intensity of the host immune response to the parasite (17). More recently, other factors such as duration (chronicity), intensity of infection (15, 36), and transmission dynamics (10, 26) have been implicated as possible determinants of disease.

Distinct immunologic responses to filarial antigens *in vitro* have been observed among the various clinical groups. Most consistent has been the finding among individuals with circulating microfilariae (29, 31) or even circulating filarial antigen (8) of a downregulated (or severely impaired) antigen-specific T-cell proliferative response and reduced production of gamma interferon (IFN- γ), a process mediated in part by interleukin-10 (IL-10) (16, 23, 32). This alteration in T-cell response can be reflected in skewed humoral responses with a preponderance of antigen-specific antibody of the immunoglobulin G4 (IgG4) isotype (11, 24).

Although the underlying mechanisms behind downregulation of cellular proliferative responses during filarial infections remain unknown, some studies have suggested a role for host macrophages (19), nitric oxide (20, 33), IL-10 (22, 28), or regulatory T cells (9, 16) in mediating this process.

The occurrence of impaired immune responses to bystander

antigens during concurrent helminth infections has been demonstrated in animal models (1, 18, 30) and in a limited number of human studies (5, 6, 34). As helminth infections, in general, are associated with a highly polarized Th2 cytokine response (4), it has been suggested that this cytokine milieu changes the manner in which responses to nonhelminth protein antigen are regulated.

It is well established that some parasitic infections lead to an impaired ability to produce antibodies against T-cell-dependent antigens. Studies examining the influence of parasitic infections on responses to bystander antigens may have relevance in reformatting the existing vaccination protocols in developing countries.

The present study was therefore designed to assess whether infection with *Wuchereria bancrofti* would alter the immune responses to tetanus toxoid (TT) following vaccination. TT was chosen because it is a potent immunogen known to induce strong T-cell-specific immune responses in humans after vaccination. Thus, it was used as a prototypical recall antigen to examine the immune responses (both cellular and humoral) in patients with various clinical syndromes associated with *W. bancrofti* infection.

MATERIALS AND METHODS

Study population. The study population was selected from individuals residing in and around Chennai, India, an area of endemicity for bancroftian filariasis. Patients were recruited through the Filariasis Control Unit under the Directorate of Public Health (Chennai) after obtaining informed consent with protocols approved by Institutional Review Boards of both the Anna University (Chennai, India) and the National Institute of Allergy and Infectious Diseases (Bethesda, Md.). Ten asymptomatic amicrofilaremic (individuals free of clinical symptoms and neg-

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ative for circulating microfilariae as determined by filtration of 1 ml of nocturnally obtained blood [endemic normal; EN], 20 microfilaremics (persons positive for circulating microfilariae and clinically asymptomatic [MF]), and 20 symptomatic amicrofilaremics (patients with chronic irreversible lymphedema of the extremities [chronic pathology; CP]) were included in this study. The levels of circulating microfilariae were determined by filtration of 1 ml of blood collected between 10 p.m. and midnight through a 3- μ m-pore-size polycarbonate filter (Nuclepore Co., Pleasanton, Calif.). Circulating filarial antigen levels were quantitated by enzyme-linked immunosorbent assay (ELISA) (TropBio, Townsville, Australia). After obtaining a patient history, a physical examination was performed, after which blood was collected from each individual for baseline studies. There were no apparent differences in the vaccination histories of the subjects studied.

Vaccination. Adsorbed TT (Serum Institute of India Ltd., Pune, India) was injected intramuscularly into the deltoid muscle in a single dose of 0.5 ml (5 flocculation [F] units of TT per dose). For the second set of studies, adsorbed TT was injected intramuscularly into the deltoid in two separate doses of 0.5 ml (5 Lf units of TT per dose) given 1 month apart.

Antigens. Saline extracts of *Brugia malayi* adult worms (BmA) were prepared as previously described (12), and soluble TT was obtained from the Massachusetts Public Health Department (Boston, Mass.).

PBMC proliferation assays. Peripheral blood mononuclear cells (PBMCs) were isolated by centrifugation over lymphocyte separation medium (Lymphoprep; Nycomed Pharma AS, Oslo, Norway). The cells were washed and plated into 96-well tissue culture plates (Costar, Corning, N.Y.) at 2×10^6 cells/ml in a volume of 200 μ l of RPMI 1640 (Sigma Chemical Corporation, St. Louis, Mo.) supplemented with 10% human AB serum (Gibco-BRL, Gaithersburg, Md.) and 80- μ g/ml gentamicin. The PBMCs were stimulated with media alone, with phytohemagglutinin (PHA; Sigma Chemical Corporation) at a concentration of 20 μ g/ml, or with TT at 0.11 Lf units/ml. All cultures were done in triplicate. The plates were incubated at 37°C and 5% CO₂ for 5 days, pulsed with 1 μ Ci of tritiated thymidine (Amersham Intl., Birmingham, United Kingdom) per well for 16 h, and harvested onto filter mats for subsequent scintillation counting. Lymphocyte proliferation was recorded as a stimulation index (SI) defined as the ratio of mean counts per minute with antigen divided by the mean counts per minute without antigen.

Cytokine assays. Separate cultures were set up for cytokine assay studies. Briefly, PBMCs were washed and plated onto 24-well flat-bottomed tissue culture plates (Costar) at 4×10^6 to 6×10^6 cells/ml in a volume of 1 ml of RPMI 1640 supplemented with 10% fetal calf serum (Gibco-BRL) and 80- μ g/ml gentamicin. The PBMCs were stimulated with or without TT at 0.11 Lf units/ml. Supernatants were harvested at 48 h and 5 days of culture and stored at -70°C before use. Capture ELISAs were used for detection of all cytokines as described previously (35); all values were derived from interpolation from standard curves run on every plate. The sensitivities of the assays for IFN- γ and IL-10 were 39 pg/ml.

TT-specific antibodies. Microtiter plates (Immulon 4; Dynatech Laboratories, Springfield, Va.) were coated with TT at a concentration of 0.56 Lf units/ml of TT per ml (for IgG and IgG isotypes) in carbonate buffer (0.045 M NaHCO₃, 0.02 M Na₂CO₃ [pH 9.6]) overnight at 4°C. After blocking the plates with blocking buffer (5% bovine serum albumin, 0.05% Tween 20 in phosphate-buffered saline [PBS]), dilutions of plasma samples in ELISA diluent (1% bovine serum albumin and 0.05% Tween 20 in PBS) were added, and the plates were incubated at 37°C for 2 h with alkaline phosphatase-conjugated goat anti-human IgG Fc (Jackson ImmunoResearch, West Grove, Pa.) for IgG or mouse ascites-derived monoclonal antibodies directed against the Fc fragment of human IgG1, IgG2, IgG3, and IgG4. Isotype-specific monoclonal antibodies (anti-IgG1 clone HP-6069, anti-IgG2 clone HP-6002, anti-IgG3 clone HP-6047, and anti-IgG4 clone HP-6023) were added at a dilution of 1:1,000, except anti-IgG2, which was added at a dilution of 1:3,000. The IgG isotype plates were subsequently incubated for 2 h at 37°C with alkaline phosphatase-conjugated goat anti-mouse IgG (Fc specific) (Jackson ImmunoResearch). *p*-Nitrophenyl phosphate in sodium carbonate buffer was used as a substrate. Plates were read on a microtiter plate reader, and unknown values were interpolated from standard curves prepared by using pooled sera from individuals with high-titer TT antibodies standardized against the World Health Organization reference serum (Statens Serum Institute, Copenhagen, Denmark) for IgG (and expressed as international units [IU] per milliliter) or pools of positive sera for each of the IgG isotypes as described previously (5). The sensitivity of the IgG assay was 0.012 IU/ml.

Statistical analysis. Statistical analysis was performed using SPSS (SPSS, Inc., Chicago, Ill.) and Statview (SAS Institute, Cary, N.C.) software. Wilcoxon signed rank test was used to compare paired data; comparison among the clinical groups was performed using the Mann-Whitney U test. Fischer's exact test was used for comparisons of response rates, and the Spearman rank correlation was used to assess relationships among variables.

TABLE 1. Prevaccination characteristics of *W. bancrofti*-infected and noninfected persons vaccinated with TT

Clinical category (n)	Median age in yr (range)	No. male/female	MF/ml range	GM circulating filarial antigen levels (AU/ml) ^a	GM TT-specific IgG antibody level in IU/ml (95% CI)
EN (10)	53 (24–60)	8/2	None	None	98 (35–277)
CP (20)	43 (23–66)	14/6	None	None	67 (23–193)
MF (20)	36 (20–48)	14/6	300–1200	7,915 (6,247–15,111)	19 (8–44)

^a AU, arbitrary units. The range is given in parentheses.

RESULTS

Study population. A total of 50 subjects was included in this study. In the first phase, 10 individuals with circulating microfilariae (MF) but with no clinical symptoms, 10 individuals with chronic lymphatic obstruction (lymphedema ranging from stages 2 to 4 [CP]), and 10 uninfected individuals living in the same area of endemicity (EN) were selected for the study. Each of these subjects was immunized with a single dose of TT, and the immune responses to TT were assessed at baseline and 3 and 6 months following vaccination. In a second phase, a separate set of 10 individuals each in the MF and CP groups was immunized with two doses of TT 1 month apart, and the immune responses were assessed prior to vaccination and at 3 and 6 months following the second vaccination. Throughout the study, none of the subjects had evidence of malaria. Results of stool examination prior to the study demonstrated no differences in intestinal pathogens among the groups; the majority of individuals showed evidence of amebic cysts (presumably nonpathogenic). Further demographic and clinical details of the study populations are given in Table 1.

PBMC proliferation to TT. It has been demonstrated that cellular responses to mitogens do not differ among the various clinical groups of lymphatic filariasis; however, parasite antigen-specific hyporesponsiveness is a characteristic feature of patent filarial infection. Indeed, while proliferative responses to BmA did not differ significantly between the CP (geometric mean [GM] SI = 7.8) and EN (GM SI = 5.8; $P < 0.1124$) groups, there was a significant difference ($P < 0.0002$) in the proliferative responses to BmA by MF lymphocytes (GM SI = 0.63) when compared to those of the other two groups (Fig. 1A). Proliferative responses to PHA were significantly higher in the CP group than those with the EN and MF groups, but responses were comparable between the EN and MF groups ($P < 0.25$) (Fig. 1B).

To assess the extent to which patent filarial infection influences the response to exogenous (nonparasitic) antigens, we next compared the cellular responses before and after vaccination with TT in the various study groups. PBMC proliferative responses to TT before vaccination were not significantly different between the EN and CP groups ($P < 0.93$), but the MF group showed significantly lower baseline proliferative responses to TT than either the EN or CP groups ($P < 0.0004$ and $P < 0.0009$, respectively) (Fig. 2A). As seen, only 1 of 10 subjects in the MF group had an SI of >2 in response to TT (GM SI = 1.25 for the group), whereas 7 of 10 in each of the other groups had an SI of >2 (GM SI = 2.52 for CP and 2.53 for EN). Six months following vaccination, the percent change in the SI from baseline levels was significantly greater in the

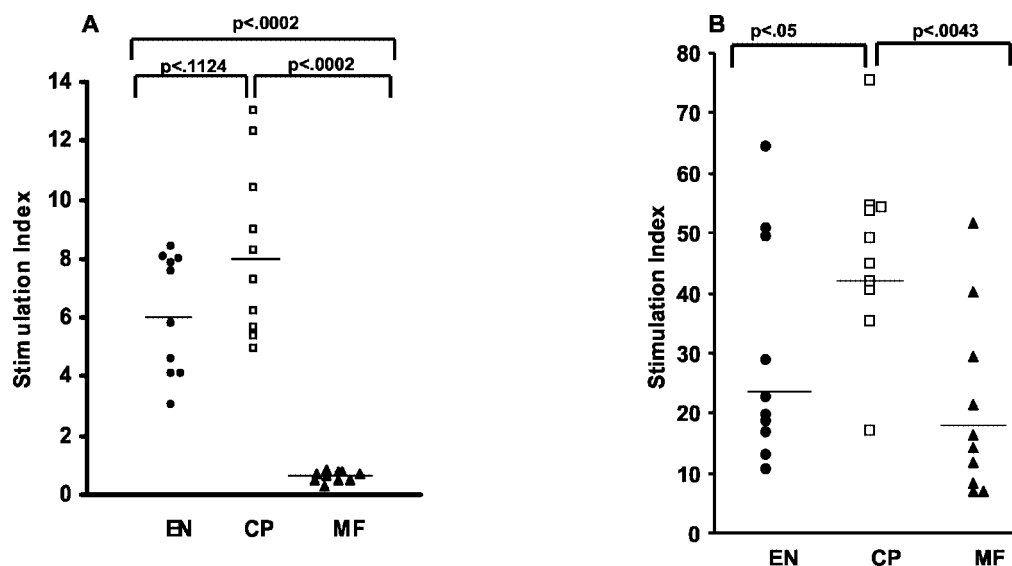


FIG. 1. In vitro PBMC proliferative responses to BmA (A) and PHA (B) in EN individuals ($n = 10$), those with lymphedema or elephantiasis (CP; $n = 10$), and those with circulating microfilariae (MF; $n = 10$). Each dot represents an individual patient. Horizontal bars represent the GM of the SI of each group.

EN and CP groups than in the MF group ($P < 0.02$ and $P < 0.03$, respectively) (Fig. 2B). The GM increases from baseline were 127% in the EN group and 107% in the CP group. This contrasted markedly with that seen in the MF group, in whom the GM increase was only 63%.

Because the baseline proliferative response to TT was low and because the TT-specific response following vaccination remained low for the MF group, a second set of 10 MF and CP subjects was immunized with two doses of the vaccine separated by a month. Results of these studies showed that pretreatment lymphocyte proliferative responses to TT were significantly greater in the new CP group compared with the MF group ($P < 0.01$). Paired-data analysis of SI of proliferative responses in CP subjects pre- and 6 months post-TT vaccina-

tion showed that at 6 months the TT-specific proliferative response increased significantly in the CP group ($P < 0.0051$), whereas there was no significant change at 6 months in the MF group ($P < 0.72$ compared with baseline) (Fig. 2C).

Cytokine production by PBMCs stimulated by TT. The levels of IFN- γ and IL-10 produced by TT-stimulated PBMCs from the different groups of subjects are shown in Fig. 3 and 4, respectively. Prior to vaccination, there was no significant difference in the levels of TT-specific IFN- γ production among the groups (Fig. 3A); however, there was a GM 400% increase in TT-specific IFN- γ from baseline levels in the EN group that differed markedly from that seen in either of the two filaria-infected groups (CP, $P < 0.05$; MF, $P < 0.0025$) at both 3 and 6 months postvaccination. There was no significant difference

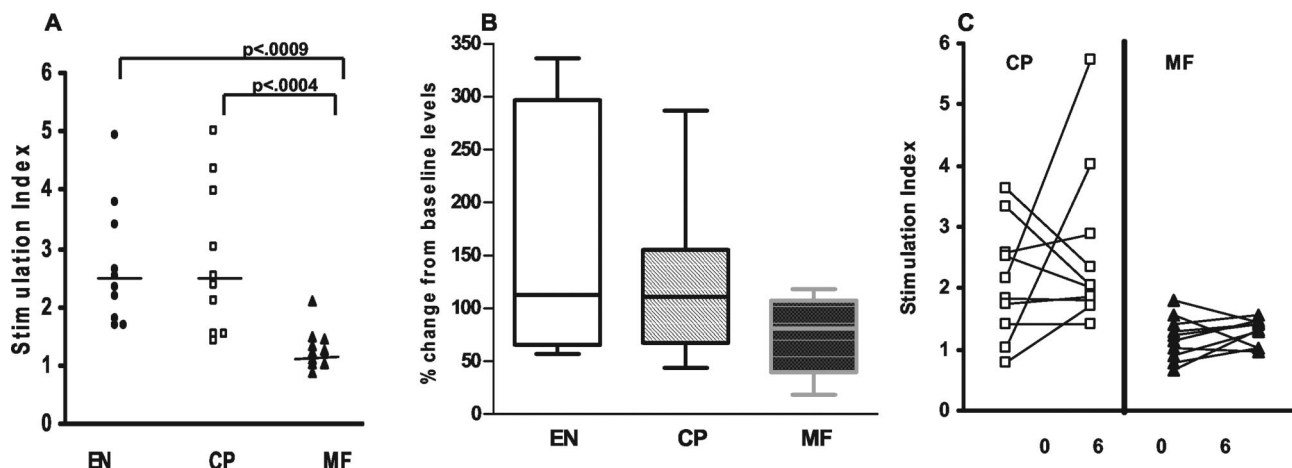


FIG. 2. In vitro PBMC proliferative responses to TT prevaccination (A), following a single TT vaccination (B), or following two vaccinations (C). (A) Each dot represents an individual; horizontal bars represent the GM SI of each group. (B) Box-and-error bar plot of the percent change in TT-specific proliferation 6 months following a single TT vaccination. The horizontal bar represents the median, boxes represent the 25th and 75th percentiles, and vertical lines represent the 95th percentile. (C) TT-specific PBMC proliferative responses expressed as SI in the CP and MF groups before and 6 months after two doses of TT.

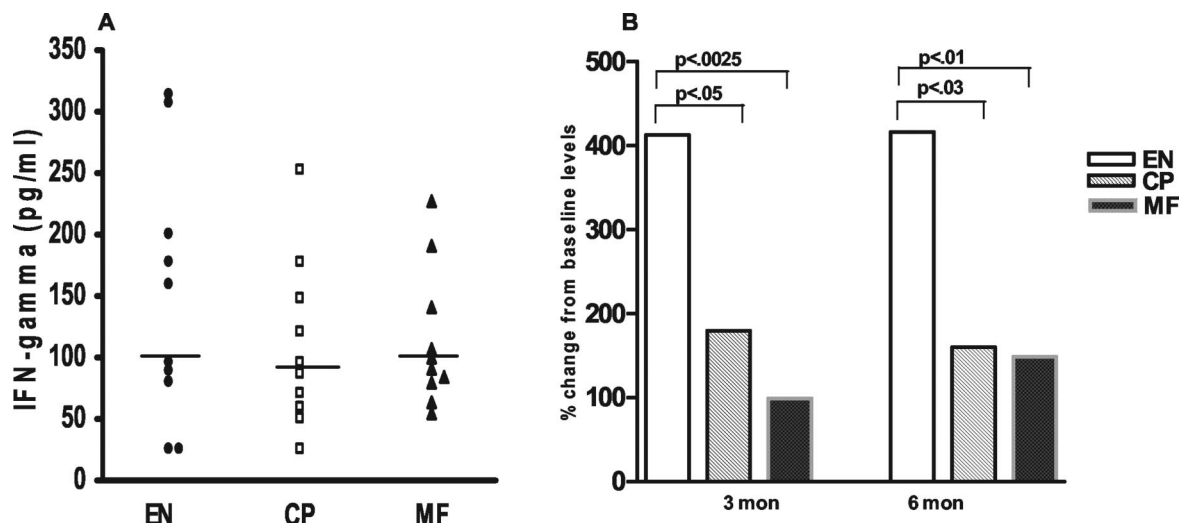


FIG. 3. TT-specific IFN- γ levels before (A) and following (B) TT vaccination. (A) Data on net production of IFN- γ in vitro, with each dot representing a single individual and horizontal bars representing the GM of the group. (B) GM percent change in TT-specific IFN- γ levels 3 and 6 months post-TT vaccination compared with prevaccination levels.

for either the CP or MF group in terms of the change in TT-specific IFN- γ production from baseline at either the 3- or 6-month postvaccination time point (Fig. 3B).

No significant difference in the levels of spontaneous IL-10 levels and those seen in response to TT was seen among the different groups before TT vaccination (Fig. 4A); however, there was a dramatic increase in the percent change of TT-specific IL-10 responses from baseline levels in the MF group compared with the EN group at 3 months post-TT vaccination ($P < 0.04$). Increases were also seen in the MF group compared with the CP group, but the difference was not statistically significant ($P < 0.1$). Subjects in the MF group still exhibited increased TT-specific IL-10 levels by 6 months postvaccination, but the percent change from baseline levels compared to the other groups was not statistically different (Fig. 4B). More interesting, however, was the inverse correlation ($P < 0.008$) noted

between the production of TT-induced IL-10 and IFN- γ in the MF group, a finding that suggests that IL-10 was, in part, responsible for the diminished IFN- γ production seen in this group.

Tetanus-specific antibody levels. To compare the antibody responses of TT by the different groups, the levels of tetanus-specific IgG and IgG isotypes (IgG1, IgG2, IgG3, and IgG4) pre- and postvaccination were measured. Fischer's exact test was used for comparison of the response rates (responders versus nonresponders) in the different study groups based on a fourfold rise in antibody titer following vaccination. Results of the antitetanus antibody responses among the groups showed that prevaccination GM levels of TT-specific IgG were significantly greater in EN subjects compared with MF subjects ($P < 0.003$), although there was no difference in the levels of TT-specific IgG between the CP and MF or CP and EN groups ($P < 0.13$ and $P < 0.93$, respectively) (Table 2).

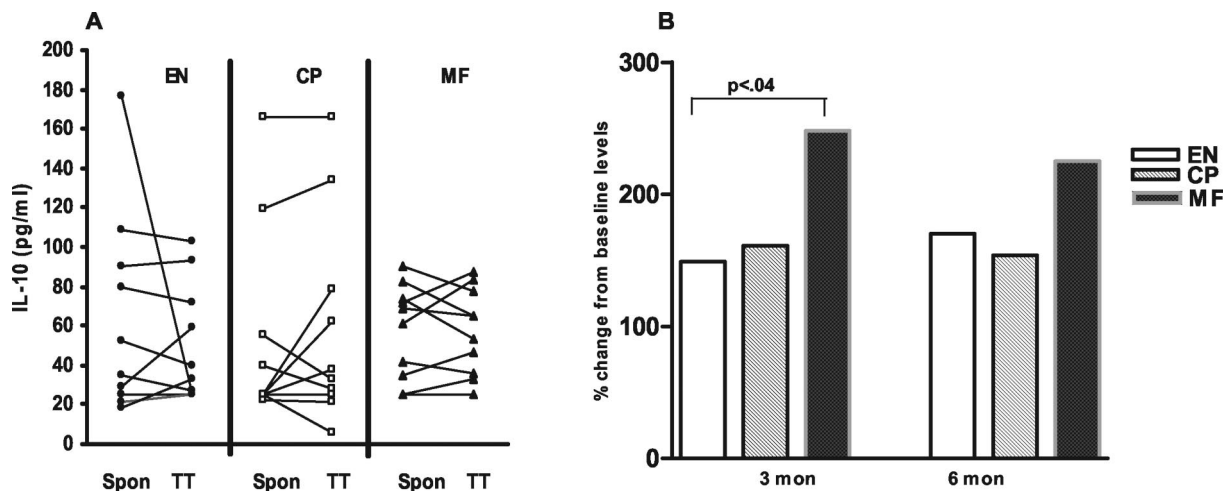


FIG. 4. IL-10 levels produced spontaneously and in response to TT prevaccination (A) and following TT vaccination (B). (A) Data on the spontaneous (Spon) and TT-specific IL-10 levels prevaccination, with each dot representing an individual. (B) GM percent change in TT-specific IL-10 levels 3 and 6 months post-TT vaccination compared with prevaccination levels.

TABLE 2. TT-specific IgG antibodies 3 and 6 months following vaccination

Clinical category (n)	IgG antibody level (GM IU/ml) ^a		
	Prevaccination	Postvaccination	
		3 mo	6 mo
EN (10)	98 (277–35)	578.1 (1,638.6–201.1)	910.2 (1,989.2–416.5)
CP (10)	67 (193–23)	320.6 (765.6–134.2)	240.6 (543.3–106.6)
MF (10)	19 (44–8.5)	66.5 (106.2–41.7)	57.1 (78.8–41.4)

^a Ranges are shown in parentheses.

Significantly greater levels of TT-specific IgG1 were seen in the EN and CP groups prevaccination compared with the MF group (EN versus MF, $P < 0.001$; CP versus MF, $P < 0.01$). GM levels of TT-specific IgG2 and IgG3 were more significant in the CP group compared with the EN and MF groups ($P < 0.0003$ and $P < 0.0032$ for EN and MF, respectively, for IgG2; $P < 0.0025$ and $P < 0.0011$ for EN and MF, respectively, for IgG3). In contrast, GM levels of TT-specific IgG4 prevaccination were more pronounced in the CP and MF groups than in the EN group ($P < 0.0002$), but no differences in TT-specific

IgG4 levels could be seen between the CP and MF groups ($P < 0.76$).

Postvaccination IgG responses to TT (expressed as percent change from baseline) were predominantly of the IgG1 and IgG4 isotypes, but no significant difference was found among the study groups in the percent changes in levels of tetanus-specific IgG and IgG1 both at 3 and 6 months postvaccination (Table 2; Fig. 5); however, increases in the levels of tetanus-specific IgG2, IgG3, and IgG4 were more prominent in the EN group than in the CP and MF groups.

DISCUSSION

Human lymphatic filariasis, a chronic disease caused primarily by the nematode parasites *W. bancrofti* and *B. malayi*, is characterized by impaired antigen-specific Th1 responses. The mechanisms underlying this impaired responsiveness are not clearly understood. Although several reports (5, 6, 34) suggest that helminth infections induce impaired cellular and humoral responses to nonparasite vaccine antigens, there has been no work done, to our knowledge, examining bystander suppression associated with lymphatic filarial infections.

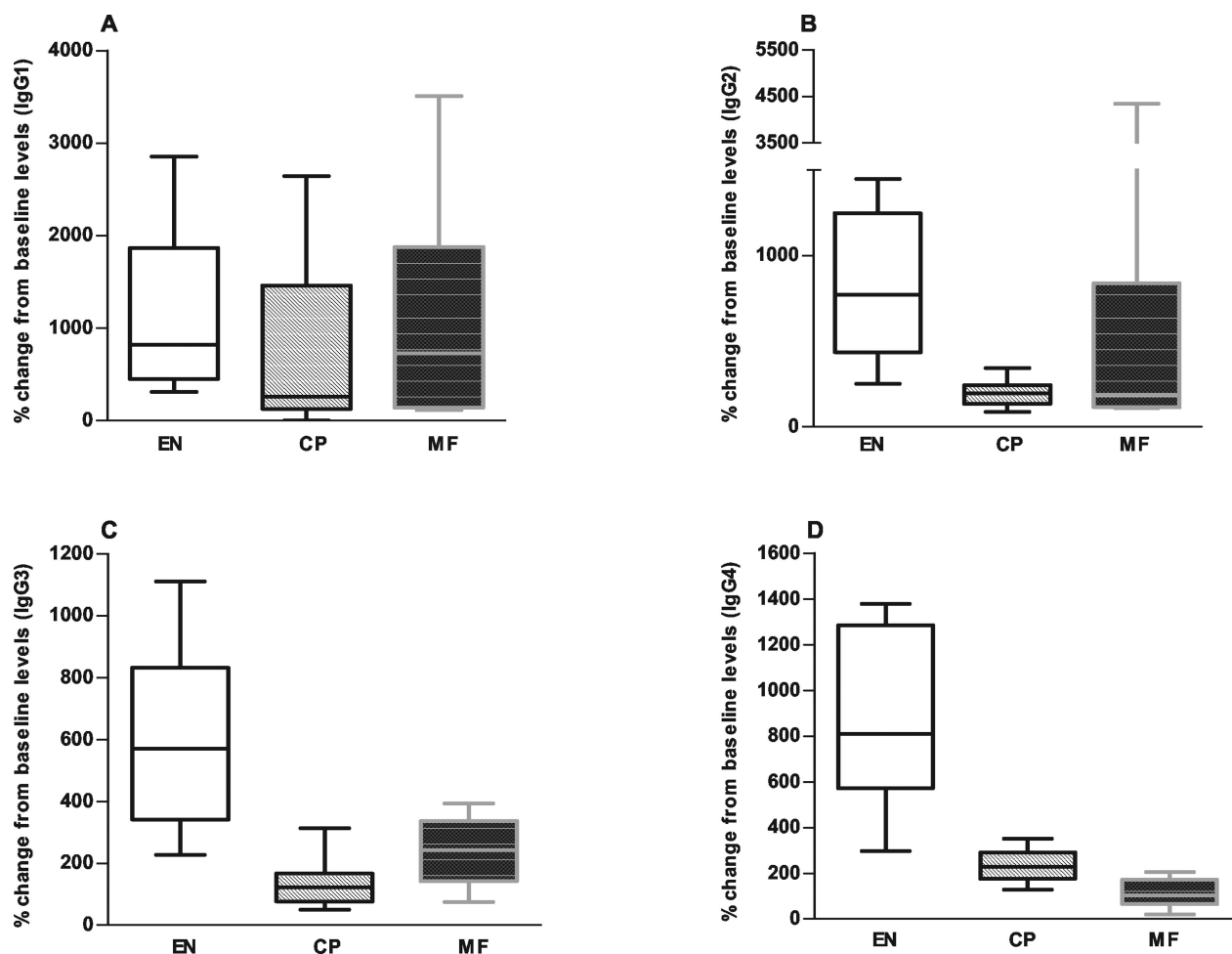


FIG. 5. Changes in antitetanus IgG isotype response levels at 6 months after TT vaccination. Data are represented as box-and-error bar plots of the percent change in TT-specific IgG1 (A), IgG2 (B), IgG3 (C), and IgG4 (D) isotypes 6 months following TT vaccination. Horizontal bar represent the median, boxes represent the 25th and 75th percentiles, and vertical lines represent the 95th percentile.

TT was chosen as the antigen used for vaccination in this study because TT is a well characterized and potent immunogen of bacterial origin known to induce long-lasting immune responses in humans. The ability to mount recall responses to TT is considered to be indicative of a healthy and intact immune system. Because the immune response to TT has been well defined, the present study intended to determine whether modulation of TT-specific immune responses occurs during filarial infection.

Antigen-specific hyporesponsiveness is the hallmark of patent filarial infections, but it leaves intact the ability to respond to mitogen (8, 22). Although stimulation with mitogens is an acceptable means of assessing general lymphocyte function, it is not always the best in vitro correlate of cellular immune responsiveness. Therefore, the present study was designed to study the TT-specific responses to evaluate an antigen-specific function in vivo. Our data demonstrated that following TT vaccination, PBMCs from the EN and CP groups proliferated normally following TT restimulation in vitro, a feature that was absent in the MF group. The fact that the MF group did not mount an appreciable proliferative response to TT may be related to the relative frequencies of responder lymphocytes or differences in the functional status of antigen-presenting cells. To determine whether those that did not respond to TT with a proliferative response were never fully sensitized during the primary vaccination series, a second set of studies with a booster immunization was carried out in an additional cohort; again, the responses to TT in the MF group were diminished. That the MF group could not mount a T-cell proliferative response to TT even after two doses of TT suggests that the lymphocytes of patients with active filarial infections, although capable of undergoing a normal response to the mitogen PHA, may have an intrinsic defect in their ability to recognize and induce a T-cell response to TT.

Although TT-specific IFN- γ levels were not significantly different among the groups prevaccination, the postvaccination IFN- γ response was markedly diminished in the filaria-infected individuals (MF and CP) compared with the EN subjects at both the 3- and 6-month time points. All groups mounted a posttreatment IL-10 response. The fact that post-TT vaccination MF PBMCs produced significantly increased levels of TT-specific IL-10 compared with the EN and CP groups suggests that the lack of tetanus-specific IFN- γ responses seen postvaccination in MF may be mediated by IL-10, a finding supported by previous studies (21, 27, 32). IL-10, in particular, has been shown to be a potent inhibitor of cellular immune responses in that it interferes with the activation of macrophages and natural killer cells as well as with the generation of IL-2- and IFN- γ -secreting Th1 cells (27). IL-10 itself has been shown to play a role in downregulation of filaria-specific responses in filaria-infected patients (24), with MF subjects having increased levels of both IL-10 protein and mRNA expression both spontaneously ex vivo and in response to parasite antigen (23). Indeed, in one study, the levels of IL-10 mRNA in MF subjects were correlated with their reduction in proliferative capacity (32).

Parasite infections such as onchocerciasis and schistosomiasis have also been associated with impaired immune responses to mycobacterial (13; A. Rougemont, M. E. Boisson-Pontal, P. G. Pontal, F. Gridel, and S. Sangare, Letter, Lancet 1:309,

1977), nonparasite (18), and vaccine antigens such as hepatitis B virus and TT (2, 5, 6, 34). Studies of cell-mediated and humoral immune responses to tetanus vaccination in patients with onchocerciasis have shown that there is a diminished cell-mediated immune response to TT vaccinations in patients with generalized onchocerciasis, although humoral responses remained unaltered (14).

Cytokine responses to nonparasite antigen have been studied in animal models with schistosome infections. It has been demonstrated in mice that infection with *Schistosoma mansoni* induces downregulation of Th1 responses and elevation of Th2 responses to unrelated nonparasite antigens such as sperm whale myoglobin as well as to parasite antigens themselves (18). Further, impairment of TT-specific Th1-like immune responses in humans infected with *S. mansoni* has been demonstrated (34). Their results suggested 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. Finally, it has been shown that impaired tetanus-specific cellular and humoral immune responses following two doses of TT vaccination occur in onchocerciasis (6), an impairment mediated in part by IL-10.

The knowledge of IgG subtype distribution is important for our understanding of the specific immune responses to infection and vaccination. In the present study, the IgG antibodies formed after TT vaccination were predominantly of the IgG1 subclass in all the clinical groups studied. This is compatible with the fact that TT, being a protein antigen, induces a T-cell-dependent B-cell response that is predominantly IgG1. We did not observe any difference in the levels of antitetanus IgG and IgG1 antibodies following vaccination among the groups studied and thus were unable to demonstrate an impaired humoral response to TT at the level of IgG1; however, EN subjects at both 3 and 6 months postvaccination had significantly higher levels of the other IgG isotypes (tetanus-specific IgG2, IgG3, and IgG4) than CP and MF subjects. Whether this is related to intensity of infection or other causes awaits clarification. Indeed, others, by stratifying onchocerca infections into heavy and light infections, have shown that heavily infected individuals tended to have more impaired humoral responses to tetanus vaccination than did individuals with light infections and uninfected controls (5). Moreover, individuals with multiple helminth infections (compared with single infections) had less of an antibody response to TT following vaccination (5) than did those with single infections. Thus, one implication from the present study is that helminth infections, in general, can modify the normal host immune response against another nonrelated antigenic stimuli and/or could modify susceptibility to other infectious agents.

MF subjects were found to respond poorly to TT secondary to defects in T-cell activation and antigen recognition. Although TT-specific antibodies are markers of the humoral arm of the immune response, defects in cellular immunity may be reflected in relatively low antibody titers. It is interesting to speculate on the role of circulating immune complexes, the presence of which might interfere with development of cellular immune responses that are essential in the defense against pathogens. The capacity of Ig to enhance or inhibit antigen-specific immune responses has been studied in some detail (7, 25) and needs further investigation in filarial infections, where the occur-

rence of circulating immune complexes in MF subjects is paralleled by a deficiency of cell-mediated immune responses to filarial antigens. Further support comes from other work (3) in which antigen-antibody complexes generated by incubating TT antigen and polyclonal antiserum inhibit secretion of IL-12 (a potent cytokine that plays an important role in development of cell-mediated immune responses) through tumor necrosis factor α -induced IL-10 and prostaglandin synthesis.

We conclude, therefore, that infection with *W. bancrofti* is associated with an impaired immune response to a vaccine antigen TT, as reflected by unresponsiveness to TT antigen in vitro and relatively impaired antibody responses. These data provide additional support for the concept that deworming and/or treatment of preexisting filarial infections may be important in any vaccine strategies employed in areas of the world where helminth infections are endemic.

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