Impaired Tetanus-Specific Cellular and Humoral Responses following Tetanus Vaccination in Human Onchocerciasis: A Possible Role for Interleukin-10

P. J. Cooper, I. Espinel, W. Paredes, R. H. Guderian, and T. B. Nutman

Onchocerca volvulus infection has been associated with impaired cellular responses to parasite antigens, an impairment that may also extend to nonparasite antigens. To investigate the mechanism of this impaired immune response, the effect of concurrent O. volvulus infection on the immune response to tetanus toxoid (TT) following tetanus vaccination was studied. The proliferative, cytokine, and antibody response to TT of O. volvulus–infected subjects (n = 19) and comparable noninfected controls (n = 20) were studied before and 6 months after vaccination with TT. Following vaccination, antibody levels, proliferative responses, and levels of interferon-γ were significantly greater in noninfected subjects (P < .05, .001, and .05, respectively); however, infected subjects produced interleukin–10, but noninfected controls did not (P < .001). These studies indicate that concurrent infection with O. volvulus can diminish the immune response to an unrelated antigen (TT) by a mechanism that is likely to involve interleukin–10.

Human helminth infections, which infect ~3 billion people worldwide, may have profound effects on the health and social development of infected children, including impaired cognition [1], growth stunting [2], and nutrient deficiencies [2]. Among these helminth parasites is Onchocerca volvulus, the causative agent of onchocerciasis or “river blindness,” which because of its chronicity and large parasite antigen burden has consequences that appear to bias the parasite-specific adaptive immune response.

Helminth infections, particularly filarial infections like onchocerciasis, are associated with cellular responses to specific parasite antigens characterized by poor lymphocyte proliferation [3, 4], impaired production of Th1 cytokines (e.g., interferon [IFN]-γ), and a relatively enhanced production of Th2 cytokines (e.g., interleukin [IL]-4 and IL-5) [5, 6]. This lack of proliferation and IFN-γ response may be related to the production of the regulatory cytokines IL-10 and transforming growth factor (TGF)-β, which may be produced in significant amounts by circulating lymphocytes from subjects infected with filarial parasites [7, 8] and which may serve to limit the pathology associated with these chronic parasitic infections.

This antigen-specific acquired cellular hyporesponsiveness may also extend to the cellular response to nonparasite antigens. Experimental animal models of infection with helminths have demonstrated that concurrent helminth infection leads to the production of type 2 cytokines in response to antigens that usually stimulate the production of type 1 cytokines [9–11]. The occurrence of a similar phenomenon in humans is unproven, although an impaired immune response to mycobacterial antigens [12, 13] and various vaccines has been reported in persons infected with both filariae [14] and schistosome [15, 16] helminths.

The demonstration of impaired immune responses to nonparasite antigens is likely to be of great public health importance, because an inappropriate immune response might increase susceptibility to infection with microparasitic pathogens, might alter the natural history and clinical presentation of the resulting disease, or might fail to provide adequate protection to vaccinees.

The present study, therefore, was designed to assess whether infection with O. volvulus would alter the immune response to a soluble protein antigen (tetanus toxoid [TT]) following vaccination.

Materials and Methods

Study population. The study was conducted in communities along the Rio Cayapas in the Santiago River Basin of Esmeraldas Province, Ecuador. All study participants were adult volunteers. O. volvulus-infected subjects were recruited from communities where the infection is hyperendemic [17]. Noninfected subjects were recruited from a nearby community where there is no disease transmission (as determined by longitudinal parasitologic and vector studies [Ecuadorian Onchocerciasis Control Programme]; Gud-erian RH, unpublished data). Inhabitants of infected and noninfected communities were of similar socioeconomic and nutritional status.

Vaccination. Adsorbed TT (gift of Pasteur Mérieux, Lyon, France) was injected intramuscularly into the deltoid in two separate 0.5-mL doses (5 LU/dose) spaced 1 month apart.

Received 15 January 1998; revised 29 April 1998.

Informed consent was obtained from all subjects, and procedures were explained in the local language. The study was done under protocols approved by NIH and Hospital Vozandes, Quito, Ecuador.

Financial support: Edna McConnell Clark Foundation.

Reprints or correspondence: Dr. P. J. Cooper, Laboratory of Parasitic Diseases, National Institutes of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland; Department of Clinical Investigations, Hospital Vozandes, Quito, Ecuador.

The Journal of Infectious Diseases 1998;178:1133–8

© 1998 by the Infectious Diseases Society of America. All rights reserved.
0022–1899/98/7804–0029$02.00
**Sample collection.** Infection status was determined by microscopic examination of skin snips for the presence of *O. volvulus* microfilariae. Skin snips were obtained by use of a corneoscleral punch from both iliac crests and incubated in saline for 24 h before examination. Skin snips negative for the presence of microfilariae were tested for the presence of *O. volvulus* DNA by use of a highly sensitive and specific polymerase chain reaction (PCR)–based assay as previously described [18].

A subject was classed as parasitologically negative if skin snips were negative by microscopic examination and by PCR before vaccination and at 1, 3, and 6 months after vaccination (after the second vaccine dose). The following samples were obtained before and 1, 3, and 6 months after vaccination: (1) 20 mL of venous blood was collected into heparinized syringes for plasma, peripheral blood mononuclear cell (PBMC) proliferation, and cytokine assays; (2) thick and thin blood films were stained with Giemsa (Sigma, St. Louis) and examined for the presence of malaria; and (3) stool samples (preserved in 10% formaldehyde and saline) were examined for the presence and quantitation of intestinal helminth eggs and larva by use of the formol-ether concentration method as previously described [19].

**PBMC proliferation assays.** PBMC were isolated by centrifugation on lymphocyte separation medium (Organon Teknika, Durham, NC). The cells were washed and plated onto 96-well flat-bottomed tissue culture plates (Corning, Cambridge, MA) at 2 x 10^6 cells/mL in a volume of 200 µL of RPMI 1640 (BioWhittaker, Walkersville, MD) supplemented with 10% human AB serum and supplemented with 1% bovine albumin and 0.05% Tween 20 in PBS). Plates were incubated at 37°C for 5 days, pulsed with 1 µCi per well of tritiated thymidine (DuPont, Boston) for 16 h, and harvested onto filter mats for subsequent scintillation counting. Results were expressed as geometric mean stimulation indices.

**Cytokine assays.** Supernatants from the PBMC proliferation assays were used to determine the levels of IFN-γ, IL-5, IL-4, and IL-10. Supernatants were harvested after 36 h and 5 days of culture and stored at -70°C before use. Capture ELISAs were used for detection of all cytokines as previously described [20]. The sensitivities of the assays for IFN-γ, IL-4, IL-5, and IL-10 were 39.1, 9.8, 3.9, and 39.1 pg/mL, respectively.

**TT-specific antibodies.** Microtiter plates (Immulon 4; Dynatech Laboratories, Springfield, VA) were coated with TT (Massachusetts Public Health Laboratory) at a concentration of 0.56 Lf units/mL in carbonate buffer (0.045 M NaHCO₃–0.02 M Na₂CO₃, pH 9.6) overnight at 4°C. 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. The plates were then incubated with alkaline phosphatase–conjugated goat anti-human IgG Fc (Jackson ImmunoResearch, West Grove, PA) for 2 h at 37°C. Plate development was done with p-nitrophenyl phosphate in sodium carbonate buffer. Plates were read on a microtiter plate reader, and unknown values were interpolated from standard curves prepared using a World Health Organization reference serum (Statens Serum Institute) and expressed as international units per milliliter. Protective levels of tetanus antitoxin were defined as those exceeding 0.15 IU/mL [21, 22]. The sensitivity of the assay was 0.01 IU/mL.

**Statistical analysis.** Skin infection intensities are expressed as the geometric mean number of microfilariae per milligram of skin. For normally distributed data (after log transformation—all proliferation and antibody results), comparisons of means from independent samples were calculated using Student’s t test, and comparisons of paired data were calculated using a paired t test. For non-normally distributed data (all cytokine results), independent samples were compared by use of the Mann-Whitney U test, and paired data were compared by use of the Wilcoxon matched-pairs signed rank sum test. Confidence intervals for non-normally distributed data were calculated by use of the Wilcoxon signed rank sum test. Bivariate analysis was done by calculation of Spearman’s rank correlation coefficients.

**Results**

**Study subjects.** Twenty noninfected and 19 infected subjects were followed at four time points (0, 1, 3, and 6 months) over 6 months. The median age (range) and the male-to-female ratio did not differ between the 2 groups (table 1). The geometric mean microfilarial intensity of the infected subjects was 13.3 microfilariae/mg. None of the noninfected subjects had *O. volvulus* microfilariae detectable in skin snips by microscopy or by PCR at any of the four observation times. None of the study subjects had evidence of infection with malaria at any of the observation times. Infection intensities of the intestinal helminths *Ascaris lumbricoides*, *Trichuris trichiura*, *Ancylostoma duodenale*, and *Strongyloides stercoralis* did not differ significantly between the 2 study groups (table 1).

**PBMC proliferation to PPD and TT.** Because cellular responses to PPD do not differ between *O. volvulus*–infected

---

**Table 1.** Characteristics of *Onchocerca volvulus*–infected and noninfected persons vaccinated with tetanus toxoid.

<table>
<thead>
<tr>
<th>Infection status</th>
<th>n</th>
<th>Age, median (range)</th>
<th>Male/female</th>
<th>Intestinal larvae (eggs)/g, geometric mean (95% confidence interval)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td><strong>Ascaris lumbricoides</strong></td>
</tr>
<tr>
<td>Noninfected</td>
<td>20</td>
<td>36 (15–75)</td>
<td>12:9</td>
<td>0 (0–0)</td>
</tr>
<tr>
<td>Infected</td>
<td>19</td>
<td>36 (17–67)</td>
<td>12:7</td>
<td>13.3 (2.5–95.5)</td>
</tr>
</tbody>
</table>

* Data are for prevaccination observations.
lated PBMC from noninfected and infected subjects are shown in table 2. Significant levels of IFN-γ (table 2) induced by PPD were produced by both subject groups, and there were no changes from baseline at each of the observation times. Both groups produced similar amounts of cytokine in response to PPD at each study time. IL-4 production was more variable among subjects in both groups (table 2); however, at all the observation times, most subjects in both groups were unable to produce IL-4 in response to PPD stimulation. Similarly, in both groups at all study times, negligible levels of IL-5 and IL-10 were produced in response to PPD.

**Cytokine production by TT-stimulated PBMC.** The cytokine responses to TT are shown in table 2. PBMC from noninfected subjects produced more IFN-γ before (\(P < .04\)) and at 6 months after (\(P < .03\)) vaccination than those from infected subjects. Levels of IFN-γ increased in both noninfected and infected study groups following TT vaccination (\(P = .02\) and \(P = .02\), respectively), but by 6 months, the levels in the noninfected group were 7-fold greater than those in the infected group.

Negligible levels of IL-4 were produced by TT-stimulated PBMC before vaccination, and these levels increased after vaccination, although there was considerable interindividual variation. Likewise, negligible levels of IL-5 were seen before vaccination, and increases in IL-5 production were seen in both study groups after vaccination (\(P = .02\) and \(P = .01\) for noninfected and infected groups, respectively). In contrast to what was seen with IFN-γ, levels of IL-10 in response to TT (negligible before vaccination in both groups) increased dramatically in PBMC from infected subjects after vaccination, whereas there was no IL-10 produced in the noninfected subjects. The difference between levels of IL-10 in the 2 groups at the 6-month time point was highly significant (\(P < .001\)). Further, IL-10 levels at 6 months were positively correlated with PBMC proliferation to TT (\(P < .01\)), suggesting that the IL-10 was actively down-regulating the proliferation and IFN-γ response to TT in the infected subjects.

**TT-specific antibody levels.** Prevaccination levels of TT-specific IgG antibodies were not significantly different between the noninfected and infected groups (figure 2A). The proportions of subjects with protective antibody levels (e.g., \(>0.15\) IU/mL) at baseline were 55% (11/20) in the noninfected group and 68% (13/19) in the infected group. This proportion increased to 100% in both groups by the 3-month observation; by 6 months after vaccination, levels had fallen below 0.15 IU/mL in 1 noninfected subject (5%) and 2 infected subjects (11%). Following vaccination, antibody levels increased in both groups (figure 2B), although at all time points, the percent increase over prevaccination levels was greater in the noninfected group. IgG levels were maximal at 3 months after vaccination in both groups. At 6 months, but not before vaccination, levels of TT-specific IgG were positively correlated with PBMC proliferation to TT (\(r = .405; P < .05\)) and negatively correlated with IL-10 levels (\(r = -.554; P < .01\)).

**Discussion**

Human helminth infections with filarial and schistosome parasites have been associated immunologically with an alteration
Table 2. Cytokine production by peripheral blood mononuclear cells stimulated with purified protein derivative of tuberculin (PPD) and tetanus toxoid (TT) before and 6 months after vaccination with TT.

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Interferon-γ</th>
<th>Interleukin-4</th>
<th>Interleukin-5</th>
<th>Interleukin-10</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before vaccination</td>
<td>6 months after vaccination</td>
<td>Before vaccination</td>
<td>6 months after vaccination</td>
</tr>
<tr>
<td>PPD</td>
<td>Noninfected</td>
<td>176 (0–1152)</td>
<td>256 (3–981)</td>
<td>0 (0–2.5)</td>
</tr>
<tr>
<td></td>
<td>Infected</td>
<td>131 (27–358)</td>
<td>453 (160–743)</td>
<td>0 (0–5.7)</td>
</tr>
<tr>
<td>TT</td>
<td>Noninfected</td>
<td>118 (0–335)</td>
<td>529 (302–872)</td>
<td>0 (0–16.6)</td>
</tr>
<tr>
<td></td>
<td>Infected</td>
<td>0 (0–9.9)</td>
<td>75 (0–453)</td>
<td>0 (0–3)</td>
</tr>
</tbody>
</table>

NOTE. Data are median cytokine levels (pg/mL) and (95% confidence intervals).

Figure 2. Tetanus toxoid (TT)–specific IgG before (A) and 1, 3, and 6 months after (B) vaccination. Columns show findings in noninfected (stippled) and infected (black) subjects. A. Baseline antibody levels represent geometric mean IU/mL of TT-specific antibodies; bars = 95% confidence intervals. B. Geometric mean % change compared with baseline levels at 1, 3, and 6 months after vaccination.
parasite antigens [7, 23, 34]. Further, the addition of anti-IL-10 neutralizing antibodies to parasite antigen–stimulated cultures partially restores cellular proliferation and increases production of IFN-γ to levels near those seen in control groups [6, 34].

TT is an efficient immunogen, which, following vaccination, induces long-lasting immunity [35]. TT is a T cell–dependent antigen that requires presentation by APCs and that activates T cells in a clonal fashion. Most TT-specific T cell clones generated from human PBMC produce both IFN-γ and IL-4 (e.g., a mixed cytokine or Th0 phenotype) [36, 37]; this parallels the findings in the present study, in which increases in the production of both type 1 (IFN-γ) and type 2 (IL-4 and IL-5) cytokines occur after tetanus vaccination. The characteristic Th0 phenotype may explain the highly responsive humoral [38] and cellular responses seen in vaccinated subjects.

At the prevaccination observation time, the cellular immune response to TT was poor in both groups and was associated with low levels of IFN-γ and negligible levels of IL-4 and IL-5 (table 2). Tetanus vaccination boosted this response in both groups, but the poor response in the infected subjects could be explained by the induction of IL-10–secreting TT-specific T cells in an IL-10–rich environment. The production of high levels of IL-10 by PBMC from O. volvulus–infected subjects—either spontaneously or in response to parasite antigens—might have two possible consequences: tolerance of TT–specific T cells and active suppression by secretion of IL–10 by TT–specific T cells.

In the first case, high constitutional production of IL-10 in the tissues at the sites of parasite sequestration (e.g., the skin and subcutaneous tissues) might induce antigen-bearing APCs that traffic to the regional lymph nodes to deliver a tolerogenic signal to T cells [39] and induce them to secrete IL-10. This would be analogous to the TGF-β–mediated suppression described in anterior chamber–associated immune deviation [40] or to bystander antigens in animal models of oral tolerance [41]. For example, antigens presented to APCs in the TGF-β–rich environment in the anterior chamber of the eye can induce them to deliver a suppressive signal to splenic antigen–specific CD8+ T cells, which are subsequently able to suppress antigen-specific T cell responses by the production of TGF-β [40].

In the second case, a suppressive signal might be delivered in the draining regional lymph nodes, where activated IL–10–secreting parasite-specific T cells are likely to be abundant, and the presence of large amounts of this cytokine might be sufficient to cause bystander effects to nonparasite antigens being presented to T cells by follicular dendritic cells. IL-10 might induce a long-lasting state of anergy [33] or generate TT-specific T cells that secrete large amounts of IL-10 (or both) [42]. Even a small number of IL–10–secreting antigen-specific T cells might be sufficient to suppress cytokine production by other TT-specific T cells.

In the current study, the humoral response to TT following vaccination was relatively impaired in infected compared with noninfected subjects (figure 2), as has been demonstrated previously [29, 30]. The reason for this is not clear but may be associated with a depression of the T cell helper function required for optimal antibody production in the presence of IL-10.

Systemic cellular responses to PPD were unaffected by O. volvulus infection in this study, and similar findings have been described previously [23, 24]. In contrast, studies of delayed-type hypersensitivity have shown reduced responses to tuberculin in O. volvulus–infected populations compared with control populations [12, 13, 29, 30]. An explanation for this discrepancy lies in the tissue distribution of the parasite. In onchocerciasis, microfilariae are restricted to the skin and subcutaneous tissues, and this distribution would likely result in an immune response restricted to the affected tissues and draining lymphatics, where microfilariae die and are destroyed in large numbers [43]. Because of this compartmentalization in the immune response, an antigen encountered in another compartment (e.g., Mycobacteria species in the respiratory mucosa) is unlikely to be influenced by parasite-specific immune responses. Therefore, systemic responses to mycobacterial antigens, for example, are unlikely to be affected, while local responses in the skin (e.g., delayed-type hypersensitivity) may be profoundly suppressed.

We have demonstrated that concurrent infection with a human helminth, O. volvulus, is associated with an impaired immune response to the exogenously administered nonparasite antigen TT, which is characterized by impaired antibody, cellular, and cytokine responses and which is associated with the antigen-specific production of IL-10.

Acknowledgments

We thank the Ecuadorian physicians, nurses, and community health workers who assisted in patient vaccination and health care provision, particularly Tamara Mancero, Raquel Lovato, Carlos Sandoval, Gregorio Montañó, Daniela Montañó, Mauricio Espinel, and Alfonso Añaña. We also thank the study communities for their cooperation and Pasteur Mérieux (Lyon, France) for the generous donation of tetanus vaccine. The assistance of Ken Farr (US Agency for International Development, Quito) is gratefully acknowledged.

References


31. delPrete G, deCarli M, Merighigogna 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.


