Clinical pathology in patients with onchocerciasis is thought to result from the cumulative effects of inflammatory reactions directed against the *Onchocerca volvulus* larvae or microfilariae that invade the skin and ocular tissues. The role of IgE-dependent mast cell activation (or type 1 [immediate] hypersensitivity) in the initiation of immune reactions against microfilarial antigens is poorly understood.

Acute inflammatory pathology resulting from microfilarial killing reactions is most easily and reproducibly observed after treatment of onchocerciasis with microfilaricidal drugs. Post-treatment reactions are caused by host inflammatory responses to microfilariae and are characterized by worsening of pruritus and rash, lymphedema, and regional lymphadenopathy. Post-treatment reactions may also be associated with systemic symptoms, such as fever, arthralgia, and postural hypotension.

Pathologic and immunologic studies of these adverse reactions (the Mazzotti reaction) have demonstrated that the onset and severity of treatment-induced reactions are associated with the development of eosinophil-rich inflammatory infiltrates at the sites of microfilarial death in the tissues [1–3] and the release of large amounts of interleukin (IL)–5 [4] and eosinophil degranulation products such as major basic protein and eosinophil-derived neurotoxin (EDN) [2, 4]. More recent studies have suggested that lipopolysaccharide-like substances released from endosymbiotic bacteria (*Wolbachia*) of filarial parasites have a role in the pathogenesis of posttreatment reactions [5–7].

We have hypothesized that drug-induced damage to microfilariae and the release of microfilarial antigens might result in IgE-dependent mast cell degranulation and the consequent development of an eosinophilic infiltrate at the sites of microfilarial destruction. To investigate the potential role of mast cells in the initiation of adverse reactions after ivermectin treatment, we examined post-treatment changes in mast cell and eosinophil numbers in skin biopsy samples from subjects infected with *O. volvulus* and changes in the plasma levels of tryptase, an indicator of mast cell degranulation, mast cell hyperplasia, or both [8]. We also investigated whether post-treatment reaction severity might be more closely associated with levels of plasma tryptase, markers of eosinophil sequestration (IL-5), and activation or degranulation (EDN) or levels of proinflammatory cytokines (tumor ne-
crosis factor [TNF]–α, IL-1β, and IL-6) as markers of Wolbachia lipopolysaccharide-induced inflammation.

Subjects and Methods

Subjects and sample collection in the Ghana study group. Local and systemic markers of mast cell function were measured in 2 separate study groups. Levels of plasma tryptase, cytokines, and eosinophil degranulation products were measured in blood samples collected from 22 adult Ghanaian men with moderate-to-heavy *O. volvulus* infection intensities who were randomly assigned in a double-blind study to receive either placebo or high-dose (400 or 600 μg/kg) ivermectin. Subject recruitment and selection has been described in detail elsewhere [4, 9]. EDTA-anticoagulated plasma samples were collected twice before treatment and at 2, 4, 6, 8, 12, 24, 36, 30, 44, and 48 h after treatment. Blood was used for collection of plasma and determination of peripheral eosinophil counts.

Subjects and sample collection in the Ecuador study group. Because skin biopsy samples were not collected during the study conducted in Ghana, punch biopsy samples collected during a separate study conducted in Ecuador were used for assessment of skin mast cell function. Samples were obtained, using 6-mm punches, from 4 Ecuadorian subjects with low *O. volvulus* infection intensities and from 1 *O. volvulus*-uninfected control subject. All biopsy samples were taken from forearm skin before administration of a standard dose (150 μg/kg) of ivermectin and at 24, 36, 40, and 48 h after treatment. Blood samples for determination of peripheral eosinophil counts were taken before ivermectin treatment and at 6, 12, 24, 36, and 48 h after treatment.

Measurement of reaction scores in the Ghana study group. Reaction scores were assessed and analyzed as described elsewhere [4, 9]. In the Ghana study group, reaction-score parameters were classified as local (pruritus, rash, gland pain, and tenderness) or systemic (fever, headache, joint or muscle pains, and lying and standing blood pressure and pulse rate). Each subject was examined for the presence of reactions at 4-h intervals during a 48-h observation period. Reaction scores were calculated as the sum of the scores for each parameter, for local or systemic parameters, and for all parameters (total reaction score) during the observation period for each subject.

Measurement of lymphedema in the Ecuador study group. Subjects were evaluated for the presence of limb lymphedema, which was determined by measurement in a standardized fashion of limb circumferences at a distance of 10 cm from a bony prominence. A score for lymphedema was calculated as the percentage change in the circumferences of all 4 limbs at each time point. The reaction parameter, lymphedema, was chosen as an index of posttreatment inflammation, because it provides a sensitive and objective clinical parameter for the assessment of local tissue inflammation.

Sample analysis. Levels of total tryptase (mature and precursor forms of α- and β-tryptases) were measured by specific sandwich ELISA [10]. Levels of mature β-tryptase, a more specific marker of mast cell degranulation, could not be determined, because some false-positive results were obtained for the samples from parasite-infected subjects (data not shown). Tissue sections were stained with Wright-Giemsa stain for eosinophil counts. Immunohistochemical detection of basophils and mast cells was done with use of the Vectastain ABC-AP kit and the Vector red substrate kit (Vector Laboratories). Monoclonal antibodies were used to detect basophils (2D7 monoclonal) and mast cells (anti-chymase B7 and anti-tryptase G3). Levels of IL-1β, IL-5, IL-10, and EDN were measured by specific sandwich ELISA [4, 11], and levels of TNF-α and IL-6 were measured by bioassays with WEHI subclone 13 and B-9 cell lines [12], respectively.

Statistical analysis. The association between 2 continuous variables was calculated using Spearman’s rank correlation coefficient. Comparison of 2 means was calculated using the Mann-Whitney U test for independent groups and the Wilcoxon signed rank sum test for paired data. Comparisons of repeated observations were done on log-transformed data by use of analysis of variance for repeated measurements. Cumulative mediator levels were calculated as the area under the curve by use of the trapezium rule.

Results

Clinical reactions and parasitologic findings in the Ghana study group. The findings for the Ghana study group have been reported elsewhere [4]. Because there were no differences in total or individual reaction-score parameters between the 8 subjects who received a 400-μg/kg dose of ivermectin and the 8 who received a 600-μg/kg dose, the data from both groups were combined into a single ivermectin treatment group for the purpose of analysis. The median ages of the placebo and ivermectin groups were 26 years (range, 22–36 years) and 32 years (range, 19–50 years), respectively. The median infection intensity was 272 microfilariae/mg (range, 64–318 microfilariae/mg) in the placebo group and 199 microfilariae/mg (range, 53–520 microfilariae/mg) in the ivermectin-treated group. Significantly greater scores for the following reaction parameters were observed in the ivermectin-treated group, compared with the placebo group, during the observation period: fever (P = .003), pruritus (P = .007), rash (P = .02), headache (P < .001), decreases in standing blood pressure (P = .001), and increases in lying (P < .001) and standing (P < .001) pulse rate. Local (P < .001), systemic (P < .001), and total (P < .001) reaction scores were greater in the ivermectin group than in the placebo group.

Clinical reactions and parasitologic findings in the Ecuador study group. This group comprised 4 *O. volvulus*-infected men with a median age of 31 years (range, 22–61 years) and 1 uninfected man aged 30 years. The median infection intensity was 3.0 microfilariae/mg (range, 1–69.5 microfilariae/mg). Although only 2 of the 4 infected subjects complained of limb swelling after treatment, all had objective evidence of increases in limb circumference after treatment that became most pronounced at 36 h (figure 1).

Changes in plasma levels of total tryptase and markers of eosinophil sequestration (IL-5) and activation or degranulation (EDN) in the Ghana study group. Pretreatment levels of total tryptase in the placebo group (geometric mean [GM], 8.7 ng/mL; 95% confidence interval [CI], 7.0–10.7 ng/mL) were equivalent to those in the ivermectin group (GM, 9.5 ng/mL; 95% CI, 8.0–11.3 ng/mL). Posttreatment tryptase levels did not
change significantly during the 48-h observation period in the placebo group. In the ivermectin-treated group, tryptase levels began to increase at 12 h after treatment ($P = .07$; figure 2); were significantly elevated, compared with those in the placebo group, by 24 h ($P < .001$); continued to increase until 30 h; and remained elevated throughout the remaining observation period ($P < .001$). Peak levels were seen at the 40-h time point: the GM absolute level was 15.4 ng/mL (95% CI, 12.6–18.7 ng/mL) for ivermectin and 8.4 ng/mL (95% CI, 7.0–11.7 ng/mL) for placebo ($P = .008$); the percentage change was 163% (95% CI, 148%–178%) for ivermectin and 105% (95% CI, 97%–112%) for placebo ($P < .001$). Changes in markers of eosinophil sequestration (IL-5) and activation or degranulation (EDN) are shown in figure 3. Significant increases in levels of IL-5 were observed at 30 h after treatment and of EDN at 24 h after treatment in the ivermectin-treated group. Levels of both IL-5 and EDN remained significantly elevated throughout the remaining observation period in the ivermectin group. Circulating eosinophil levels had decreased by 66% (95% CI, 53%–77%) at 24 h after treatment (figure 4B). Peripheral eosinophil counts remained low throughout the remaining observation period. During the 48-h observation period, levels of plasma tryptase ($P < .0001$), IL-5 ($P < .0001$), and EDN ($P < .0001$) were higher and peripheral eosinophil counts ($P < .0001$) were lower in the ivermectin-treated group than in the placebo group (by analysis of variance).

Changes in plasma levels of pro- and anti-inflammatory cytokines in the Ghana study group. There were no significant changes in levels of TNF-$\alpha$, IL-1$\beta$, and IL-10 during the 48-h observation period in the ivermectin-treated group (figure 3). Although levels of IL-6 were significantly elevated at several posttreatment time points (figure 3), there was no evidence of an overall significant difference in IL-6 levels between the 2 study groups (by analysis of variance).

Correlations between tryptase levels and reaction scores. Strong positive correlations were observed between cumulative plasma tryptase levels and the reaction-score parameters of rash ($r = 0.59; P = .009$), fever ($r = 0.51; P = .02$), muscle ache ($r = 0.44; P = .05$), joint pain ($r = 0.63; P = .005$), headache ($r = 0.58; P = .009$), and postural decreases in blood pressure ($r = 0.47; P = .04$) and pulse rate ($r = 0.75; P = .0008$). Plasma tryptase levels were strongly correlated with local ($r = 0.61; P = .006$), systemic ($r = 0.76; P = .0007$), and total reaction scores ($r = 0.69; P = .002$). In addition, cumulative levels of plasma tryptase were associated with levels of EDN ($r = 0.69; P = .006$), IL-5 ($r = 0.65; P = .004$), and peripheral eosinopenia ($r = -0.63; P = .005$).

Correlations between other mediator levels and reaction scores in the Ghana study group. Correlations between cumulative cytokine levels and local, systemic, and total reaction scores are shown in table 1. Cumulative levels of IL-6 were correlated with systemic reaction scores ($P = .02$), but levels of IL-1$\beta$, TNF-$\alpha$, and IL-10 were not. Strong correlations were observed between systemic reaction scores and cumulative levels of EDN ($P = .003$) and IL-5 ($P = .004$). Local reaction scores were correlated with cumulative levels of EDN ($P = .01$).

Changes in skin eosinophil, mast cell, and basophil counts in the Ecuador study group. Before treatment, few eosinophils were observed in skin biopsy samples from the 4 infected Ecuadorian subjects. Circulating eosinophil levels decreased by 63% (range, 23%–79%) by 24 h after treatment and remained low throughout the remaining observation period (figure 4B). An increase in dermal eosinophil numbers was noted at 36 h after treatment (figure 1). In the 4 infected subjects, mast cell counts increased after treatment, with a transient peak at 24 h (figure 1). Basophils were observed in 1 skin biopsy sample obtained from a subject at 36 h after treatment but not in any other biopsy sample obtained during pre- or posttreatment time points. The transient peak in mast cell counts at 24 h after treatment preceded the development of both dermal eosinophilia and peak tissue inflammation (i.e., lymphedema). All mast cells were chymase positive and resided in the dermis. No eosinophils or basophils were observed in any of the skin biopsy samples from the uninfected control subject, and mast cell counts did not change during the observation period in this subject.

Discussion

Although the role of mast cells in the initiation of allergic inflammation is well documented [13], few studies have examined the role of these cells in the initiation of inflammatory reactions directed against the larvae of tissue helminths. A good model to investigate such a potential role is provided by the
inflammatory response to *O. volvulus* microfilariae after treatment with ivermectin. This reaction is associated with development of clinically relevant adverse reactions [4, 9, 12] coincident with development of an intense eosinophil-rich inflammatory infiltrate at the tissue sites of microfilarial death and destruction [2, 3] and local expression of eosinophil chemoattractant chemokines [14].

**α**- and **β**-tryptase genes encode serine proteases that are selectively produced by mast cells, much smaller amounts being found in peripheral blood basophils [15]. Mature **β**-tryptase is stored in the secretory granules of human mast cells and is released, together with histamine and other preformed mediators, during activation-induced degranulation. In contrast, **α**- and possibly **β**-tryptase precursors are spontaneously secreted by mast cells [16, 17] and perhaps basophils. In serum or plasma from healthy individuals, levels of **β**-tryptase are undetectable (<1 ng/mL), whereas total tryptase levels range from 1 to 15 ng/mL. Elevated total plasma tryptase levels in plasma samples from individuals with non–acute phase infection are associated with systemic mastocytosis and with some cases of acute myeloblastic leukemia, whereas increases in levels during short observation periods (minutes) are typical of systemic anaphylaxis [8, 10, 18, 19].

To investigate the potential role of mast cells in the adverse reactions that follow the treatment of onchocerciasis with ivermectin, we measured changes in mast cell numbers and in levels of total plasma tryptase after ivermectin treatment. The study was done with 2 groups of *O. volvulus*-infected subjects, a group of subjects from Ecuador, who received a standard dose (150 μg/kg) of ivermectin and a group of subjects from Ghana, who received a high dose (400 or 600 μg/kg) of ivermectin. The group of subjects treated with high-dose ivermectin was chosen for comparison with placebo, because we have demonstrated elsewhere, in the same group of subjects, that high-dose ivermectin treatment induces earlier onset and more-severe adverse reactions than does the standard dose [4].

In this study, we were able to demonstrate marked increases in plasma tryptase levels as a consequence of ivermectin-associated microfilarial killing. The changes in plasma tryptase levels occurred early, and levels were elevated by 12 h after treatment. The increase in plasma tryptase levels preceded the development of adverse reactions, which started after 24 h and peaked between 32 and 36 h after treatment, in this study group [9]. The increase in plasma tryptase occurred at approximately the same time as the decrease in circulating eosinophil counts and release into the peripheral circulation of the eosinophil degranulation product EDN. Furthermore, changes in plasma tryptase levels were correlated strongly with reaction scores (local, systemic, and total), markers of eosinophil sequestration (peripheral eosinopenia and plasma IL-5), and activation or degranulation (plasma EDN).

The strong relationship between the onset and severity of ivermectin-associated adverse reactions and the release of mast cell tryptase into the peripheral circulation supports a role for mast cell hyperplasia and/or degranulation in the early inflammatory response to *O. volvulus* microfilariae that are killed or damaged after treatment. Mast cells may have a role in the initiation of the inflammatory cascade, leading to eosinophil recruitment in the tissues. Such an initiating event might be the release of parasite antigens from ivermectin-damaged microfilariae.
lariae, leading to IgE-dependent mast cell degranulation. Our observations of a transient increase in mast cell numbers (at 24 h after treatment) before development of dermal eosinophilia and significant clinical inflammation (e.g., lymphedema) would suggest that mast cells play a role as initiators of treatment-associated inflammation. The increase in mast cell density may have followed recruitment of circulating precursor cells, local proliferation of resident mast cells, or migration of mature cells from adjacent tissues [20]. The rapid decrease in mast cell numbers by 36 h after treatment may have resulted from apoptosis or from failure to detect degranulated mast cells (depleted of tryptase) [2]. Failure to detect basophils in these treatment-associated skin lesions contrasts with late-phase responses to intracutaneous allergen administration [21, 22], which suggests that the inflammation occurring after antiparasite treatment is not a classic IgE-dependent late-phase response.

A number of reported observations suggest that mast cell activation may be the primary initiating event in the inflammatory reactions associated with onchocerciasis. First, in onchocercal nodules, perivascular mast cells are found in close proximity to nongravid female parasites, whereas nodules containing gravid or microfilariae-releasing female parasites are surrounded by an infiltrate containing both eosinophils and mast cells, indicating that eosinophil recruitment is mast cell dependent [23, 24]. Second, after treatment of onchocerciasis with the microfilaricidal drug diethylcarbamazine, mast cell degranulation is maximal at the time of the appearance of activated eosinophils in the skin [2]. Third, patients with hyperreactive onchocerciasis, or sowdah, have much greater numbers of activated nodular mast cells that stain strongly for IgE than do patients with generalized onchocerciasis [25]; immediate hypersensitivity reactions, therefore, may have a role in the enhanced inflammatory pathology associated with this rare manifestation of infection.

Elevated plasma tryptase levels could reflect either regulated release of tryptase by degranulation of mast cells or spontaneous release of this molecule by a large mast cell biomass, as occurs in mastocytosis [8, 10, 15]. The transient nature of tissue mastocytosis in the face of persistent elevation in plasma tryptase levels after ivermectin treatment suggests that these elevated levels reflect a combination of mast cell hyperplasia and prolonged mast cell degranulation.

The strong correlations between cumulative levels of plasma tryptase and the severity of local and systemic posttreatment reactions suggest that IgE-mediated inflammation plays a role. Local reactions, such as pruritus and rash, were strongly correlated with plasma tryptase and EDN levels in the Ghana study group. Development of lymphedema in the Ecuadorian subjects was closely related to development of tissue eosinophilia and a decrease in the density of dermal mast cells. These data suggest that mast cell degranulation and hyperplasia are important precursors to development of localized inflammation after ivermectin treatment.

Systemic posttreatment reactions could be caused by either a systemic inflammatory response to the presence of endotoxin-like substances released by Wolbachia endosymbionts [5–7] or type I hypersensitivity–induced anaphylaxis to the presence of parasite allergens. Systemic reactions were strongly associated
with levels of plasma tryptase, IL-5, and EDN, which indicates that the latter is the more probable explanation; however, a contribution of the former cannot be excluded.

A recent study that included some individuals in the Ghana group of the present study showed a significant correlation between levels of Wolbachia DNA and TNF-α in serum and a correlation between peak DNA levels and reaction scores [7]. The association between plasma IL-6 and the severity of systemic reactions might provide additional support, although circulating IL-6 is induced nonspecifically by acute inflammatory stimuli [26].

We were not able to demonstrate a change in circulating TNF-α levels during the observation period or an association between TNF-α levels and the severity of systemic reactions. Other studies have shown that O. volvulus extracts can induce TNF-α and IL-8 production by monocytes [27] and that treatment of onchocerciasis with microfilaricidal drugs is associated with posttreatment changes in levels of TNF-α [12, 28] but not IL-8 [12]. Our data suggest that the release of lipopolysaccharide-like substances from Wolbachia endosymbionts [5] of filarial parasites contribute only modestly to the systemic posttreatment reactions observed in this study group.

Although the 2 study groups were different with respect to ivermectin dose and skin microfilarial intensities (mean of 3 microfilariae/mg in the Ecuador study group vs. 197 microfilariae/mg in the Ghana study group), we would argue that the observations made in the Ecuador study group can be generalized to the Ghana study group (and vice versa), because the onset of posttreatment reactions in the Ecuador (figure 1) and Ghana [9] study groups occurred at approximately the same time (after 24 h) and were maximal at 36 h and because the magnitude of the decrease in peripheral blood eosinophil counts was remarkably similar in both study groups (figure 4) at the time of maximal eosinopenia (i.e., 24 h; 66% reduction in the Ghana study group, vs. 63% in the Ecuador study group). Other studies have suggested that the severity of posttreatment reactions after ivermectin treatment is related to pretreatment microfilarial intensity [12] and that higher-dose ivermectin treatment is associated with a more profound peripheral eosinopenia [4] and higher circulating levels of IL-5 and EDN [4] than is standard-dose treatment. The timing and magnitude of eosinophil sequestration and activation, however, are likely to have been similar in both study groups. The consistency in the results of the 2 study groups—showing changes in dermal mast cell numbers (in the Ecuador study group) and mast cell hyperplasia and degranulation (in the Ghana study group) that appeared to be associated with eosinophil-mediated reactions—provides strong support for the study conclusions.

In conclusion, we have demonstrated that treatment of onchocerciasis with high-dose ivermectin is associated with marked posttreatment increases in plasma tryptase levels, which are, in

Table 1. Spearman’s rank correlation coefficients for associations between plasma levels of immune mediators and local, systemic, and total reaction scores in 22 O. volvulus-infected subjects from Ghana who received 400/600μg/kg ivermectin or placebo.

<table>
<thead>
<tr>
<th>Immune mediator</th>
<th>Local</th>
<th>Systemic</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α</td>
<td>0.04</td>
<td>0.07</td>
<td>−0.22</td>
</tr>
<tr>
<td>IL-1β</td>
<td>−0.21</td>
<td>−0.23</td>
<td>−0.39</td>
</tr>
<tr>
<td>IL-5</td>
<td>0.26</td>
<td>0.64a</td>
<td>0.57a</td>
</tr>
<tr>
<td>IL-6</td>
<td>0.25</td>
<td>0.52b</td>
<td>0.38</td>
</tr>
<tr>
<td>EDN</td>
<td>0.58b</td>
<td>0.71*</td>
<td>0.61a</td>
</tr>
<tr>
<td>Tryptase</td>
<td>0.61a</td>
<td>0.76b</td>
<td>0.69*</td>
</tr>
</tbody>
</table>

NOTE: EDN, eosinophil-derived neurotoxin; IL, interleukin; TNF, tumor necrosis factor.

* P < .01

b P < .05

c P < .001
turn, associated with a decrease in peripheral eosinophil counts and increases in the levels of peripheral markers of eosinophil sequestration and activation. We showed in a separate group of subjects, who received a standard dose of ivermectin, that mast cell density increased soon after treatment but decreased by the time of onset of dermal eosinophilia and clinical inflammation. Localized posttreatment reactions were strongly correlated with levels of plasma tryptase and EDN, whereas systemic reactions were correlated with levels of plasma tryptase, EDN, IL-5, and IL-6. No associations between levels of proinflammatory cytokines IL-1β and TNF-α and the severity of systemic posttreatment reactions were observed. These findings indicate that mast cell recruitment, activation, and degranulation probably have an important early role in development of inflammatory reactions directed against *O. volvulus* microfilariae. The data suggest that both local and systemic posttreatment reactions have a primary allergic component related to microfilarial destruction in the tissues.

**Acknowledgment**

We thank Brenda Rae Marshall for editorial assistance.

**References**


