CONCISE COMMUNICATIONS

Eotaxin Expression in *Onchocerca volvulus*–Induced Dermatitis after Topical Application of Diethylcarbamazine

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In persons with onchocerciasis, topical application of the anthelminthic diethylcarbamazine (DEC) induces clinical and histologic responses similar to acute papular onchodermatitis, including recruitment of eosinophils to the skin. To determine whether the eosinophil chemokine eotaxin is likely to be associated with eosinophil recruitment in onchodermatitis, DEC was applied to a 5-cm² area on the skin of infected persons, and biopsies were taken from lesions 24 h later. Histologic analysis showed elevated dermal and epidermal eosinophils compared with tissue from an adjacent (untreated) site. Reverse transcription–polymerase chain reaction showed that eotaxin gene expression in DEC-treated skin was elevated 2- to 17-fold compared with control tissue. Eotaxin immunoreactivity was noted in mononuclear cells and eosinophils in the perivascular region of the dermis and in lymphatic and vascular endothelial cells. Together, these observations are consistent with a role for eotaxin in recruitment of eosinophils to the dermis in early stage onchocercal skin disease.

Skin disease occurring after infection with the parasitic helminth *Onchocerca volvulus* is a major public health problem throughout west and central Africa and was an important factor in establishing the African Program for Onchocerciasis Control [1–3]. Recent reports indicate that of 15 million infected persons in Africa outside the control program area, about 8.6 million reside in areas in which the parasite strains are associated with severe skin disease [1–3]. Although there are several forms of onchocerbal skin disease, acute papular onchodermatitis is one of the earliest pathologic manifestations [4, 5]. The papular rash is intensely pruritic and the itching results in such severe distress, sleep loss, and physical disfigurement that it has a serious social and economic impact on infected communities [2, 3]. Normal migration of *O. volvulus* larvae (microfilariae) through the dermis does not elicit an inflammatory response. However, larval death occurring as a result of either natural attrition or after chemotherapy stimulates an intense inflammatory response that appears to mediate tissue destruction [4, 6, 7]. Eosinophils are a prominent component of the inflammatory infiltrate, and deposition of eosinophil granule proteins on cutaneous tissue is likely to contribute to development of the clinical symptoms [7, 8]. As a first step in identifying specific mediators of eosinophil migration to the skin in onchodermatitis, we examined expression of the β-chemokine eotaxin, which has potent and specific activity for eosinophils [9, 10].

Methods

Study population. The study population was composed of males and females aged 5–15 years from the village of Gnonkoradji on the Sassandra River in southwestern Côte d’Ivoire. We applied 10% diethylcarbamazine (DEC) in Nivea lotion (Beiersdorf, Norwalk, CT) to a 5-cm² area above the right ileac crest, which was covered with a sterile dressing to prevent scratching. Control patches containing lotion alone were applied to the left ileac crest. The dressings were removed 24 h later, and a positive response was determined by the appearance of raised papules. Sixteen persons with an intensely positive response were chosen for the study. (The results of the DEC “patch test” reactions in this population will be reported separately in the context of a World Health Organization epidemiologic survey of the region.) A single 2-mg biopsy was taken from a lesion on DEC-treated skin and from the control site using a sterile sclerodermal punch. Paired DEC-treated and control tissue were either fixed in formalin for immunohistochemical analysis or snap frozen in liquid N₂ for reverse transcription–polymerase chain reaction (PCR). This population had been treated the previous year with the microfilaricidal ivermectin (Mectizan; Merck Sharpe & Dohme, Rahway, NJ).
Figure 1. Clinical and histologic responses in persons infected with parasitic helminth Onchocerca volvulus after topical application of diethylcarbamazine (DEC). Patients from an area endemic for onchocerciasis received a topical application of DEC in lotion on a 5-cm² area on the iliac crest. Control sites on the same subject were exposed to lotion alone. After 24 h, the responses at both sites were assessed, skin biopsy specimens from representative patients were fixed in formalin, and sections were immunostained with antibodies to eosinophil major basic protein (MBP; B–F) or to eotaxin (G–J). A, In heavily infected persons, numerous papules are visible that are localized to the site of DEC application. No papules were detected after exposure to lotion alone. B, Epidermal lesion in DEC-treated skin after immunostaining with antisera to eosinophil MBP and visualized with Vector red. Arrow, O. volvulus microfilariae (original magnification, ×400.) C, D. Dermal lesion with eosinophils adjacent to microfilaria after DEC treatment. Note disruption of dermal/epidermal barrier near worm (original magnifications, ×100 [C], ×400 [D]). E, F. Skin of control lotion-treated skin showing worm in dermis with normal architecture and no apparent inflammatory response (original magnifications, ×100 [E], ×400 [F]). G–J. Eotaxin expression in DEC-treated skin (visualized with Vector red). G, Mononuclear cells around blood vessels in dermis. Note reactivity with inflammatory cells but not vascular endothelial cells. H, I. Eotaxin reactivity in perivascular eosinophils (arrowheads) with variable immunostaining of vascular endothelial cells. J. Lymphatic endothelial cells stain positively for eotaxin.
Immunohistochemistry. Formalin-fixed tissue was processed and embedded in paraffin by standard methods. For detection of eosinophils, 5-μm sections were immunostained with rabbit antisera raised against purified human eosinophil major basic protein (MBP; diluted 1:1000). For detection of eotaxin, we utilized affinity purified rabbit sera to recombinant eotaxin [11]. Antibody reactivity was visualized using Vector red, as described elsewhere [12].

Eotaxin gene expression. RNA was extracted using Qiagen reagents according to the manufacturer’s directions (Qiagen, Chatsworth, CA) and reverse transcribed using reverse transcriptase (Superscript II; Gibco BRL, Gaithersburg, MD). Copy DNA was then amplified by PCR at 95°C, 1 min, 65°C, 1 min, and 72°C, 2 min, using the following oligonucleotides: β-actin: 5’-TGACGGGTCAACCCACACTGTGCCCATCTA, 3’-CTGAGGACTTGCGGTTGACGGGGTC (27 cycles); eotaxin: 5’-TGCGAGACCCTAAGAAAGAGT, 3’-CAGGAGAAGAGAGGAGGAG (38 cycles). PCR products were electrophoresed in a 1.5% agarose gel and stained with SYBR green (Molecular Probes, Eugene, OR). Gels were then scanned using a Storm scanner and ImageQuant software for densitometric analysis (Molecular Dynamics, Sunnyvale, CA).

Results

DEC-induced papule formation in O. volvulus–infected persons. Topical application of DEC in lotion has been shown to induce papule formation in infected persons [13, 14]. In the current study, raised papules detected after 24 h were localized to the area of DEC administration (figure 1A). No response was seen after exposure of infected persons to lotion alone or in uninfected Africans and North Americans exposed to DEC under identical conditions (not shown).

Elevated dermal and epidermal eosinophilia after topical DEC. To assess the distribution of eosinophils in the skin, biopsies were immunostained with antibody to eosinophil MBP. As shown in figure 1B, eosinophils were present in epidermal abscesses, often in close proximity to the parasites. Eosinophils were also present throughout the dermis of DEC-treated skin (figure 1C, 1D). In contrast, no inflammation around the parasites was detected in skin exposed to lotion alone, and eosinophils were rarely detected (figure 1E, 1F).

Elevated eotaxin expression in response to DEC. Given the presence of eosinophils in the skin of infected subjects after DEC treatment, we next determined whether expression of eotaxin was elevated in DEC-treated skin. As shown in figure 2A, eotaxin gene expression was elevated in 7 of 8 DEC-treated tissues compared with untreated tissue. Densitometric analysis showed a 2- to 17-fold increase in DEC-treated skin compared with skin exposed to lotion alone (figure 2B).

To determine which cells in the skin express eotaxin, tissue sections were immunostained with antisera to human eosinota. As shown in figure 1G–1J, mononuclear cells and eosinophils in the perivascular region of the dermis reacted strongly with this antibody. Vascular endothelial cells had inconsistent reactivity, with no staining in some persons (figure 1G) and weak immunostaining in others (figure 1H, 1I). Lymphatic vessels were also a source of eotaxin (figure 1I). Keratinocytes did not produce eotaxin based on immunoreactivity, and no eotaxin staining was detected in tissue biopsies of untreated skin or on DEC-treated skin from uninfected persons (not shown).

Discussion

Although several chemotactic cytokines have activity for eosinophils, we focused on eotaxin because this chemokine can directly mediate eosinophil recruitment to the skin [10] and because our previous studies indicated an important role for eotaxin in eosinophil recruitment in O. volvulus–mediated corneal pathology [9].

In the current study, we found that eotaxin gene expression was elevated in DEC-treated skin, compared with untreated skin. However, low-level eotaxin expression was detected in some of the untreated biopsies, consistent with the low constitutive eotaxin mRNA levels expressed by human dermal fibroblasts in vitro [15]. As only a single biopsy was taken from control and DEC-treated sites from each subject, we were unable to determine whether there was a direct relation between eotaxin mRNA and protein expression. However, taken together, the observations that eotaxin gene expression was elevated after DEC treatment compared with control tissue from the same subject and that antisera to eotaxin was more reactive in DEC-treated skin than control skin indicate that eotaxin expression is elevated at the site of inflammation.

In addition to eosinophils, neutrophils are prominent in DEC-induced skin lesions in infected persons [14] and in a
murine model of onchocercal dermatitis [12]. Future studies will therefore examine the expression of other eosinophil and neutrophil chemokines in DEC-induced onchodermatitis. In addition, the current study population was treated with ivermectin the previous year and was therefore likely to have fewer microfilariae in the skin than would untreated persons. Continuing studies will examine untreated persons who are likely to have a more intense response to DEC.

In summary, the current study provides evidence for a possible role for eotaxin in helminth-mediated dermatitis. An understanding of these molecular events and identification of the key molecules involved in eosinophil recruitment may then suggest therapeutic approaches that specifically inhibit eosinophil accumulation and therefore prevent eosinophil-mediated damage in the tissue. Results from these studies may also have implications for other forms of dermatitis, such as atopic dermatitis, in which eosinophils are important mediators of inflammation.

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References