CD4\(^+\) Depletion Selectively Inhibits Eosinophil Recruitment to the Cornea and Abrogates *Onchocerca volvulus* Keratitis (River Blindness)

LAURIE R. HALL, JUSSF T. KAIFI, EUGENIA DIACONU, AND ERIC PEARLMAN

Departments of Medicine and Ophthalmology, Division of Geographic Medicine, Case Western Reserve University and University Hospitals of Cleveland, Cleveland, Ohio 44106

Received 1 May 2000/Accepted 10 June 2000

Previous studies demonstrated that in the murine model of *Onchocerca volvulus* keratitis, neutrophils and eosinophils are recruited into the cornea in a biphasic manner in response to intrastromal injection. To determine if CD4\(^+\) T cells regulate migration of neutrophils and eosinophils into the cornea, CD4\(^+\) cells were depleted using monoclonal antibody GK1.5 before intrastromal injection of parasite antigens. Depletion of CD4\(^+\) cells abrogated corneal opacification at later but not early stages of disease. Consistent with this observation, CD4 depletion significantly impaired recruitment of eosinophils to the cornea but had no effect on neutrophils. These data indicate that CD4\(^+\) T cells mediate sustained *O. volvulus* keratitis by regulating eosinophil recruitment to the cornea.

Although the World Health Organization has reduced the prevalence of onchocerciasis in 11 countries in West Africa, ocular onchocerciasis (river blindness) remains a leading cause of infectious blindness and severe visual impairment throughout sub-Saharan Africa (5, 12, 16). To examine the inflammatory events that lead to *Onchocerca volvulus*-mediated corneal disease (keratitis), we have developed a murine model in which repeated immunization with *O. volvulus* antigens leads to the selective induction of a Th2 response. This response is characterized by CD4-dependent interleukin-4 (IL-4) and IL-5 production (10), elevated blood eosinophils, and parasite-specific immunoglobulin E (IgE) and IgG\(_1\) (reviewed in reference 3). Subsequent intrastromal injection of *O. volvulus* antigens leads to biphasic recruitment of neutrophils and eosinophils into the cornea and development of corneal opacification (3, 9).

It has been shown previously that athymic nu/nu mice do not develop keratitis when immunized and challenged intrastromally with *O. volvulus*, although disease can be reconstituted by transferring spleen cells from immunized mice (10). Although these observations clearly demonstrate that T cells are essential for the development of *O. volvulus* keratitis, these studies did not differentiate between the role of T cells in the development of systemic responses to parasite antigens and a possible role for T cells in regulating migration of neutrophils and eosinophils into the cornea. The purpose of the current study was to determine if T cells, in addition to being essential for development of systemic responses, also regulate the recruitment of neutrophils and eosinophils into the cornea.

C57Bl/6 mice (Charles River Laboratories, Wilmington, Mass.) received three weekly subcutaneous immunizations with 10 \(\mu\)g of *O. volvulus* antigens in a 1:1 ratio with adjuvant containing squalene (Aldrich Chemical, Milwaukee, Wis.), Tween 80 (Fisher, Fair Lawn, N.J.), and pluronic acid (BASF, Parsippany, N.J.). Mice were depleted of CD4\(^+\) cells using rat anti-mouse CD4 (GK1.5) according to the protocol shown in Fig. 1. This regimen consistently depleted mice of \(>94\%\) of CD4\(^+\) cells as determined by fluorescence-activated cell sorter analysis (19% compared with 1% in anti-CD4-treated animals). Control mice were injected with rat IgG. For intrastromal injections, a gas-tight syringe (Hamilton, Reno, Nev.) was used to inject 5 \(\mu\)g of *O. volvulus* antigens as described previously (10).

We first determined the effect of CD4 depletion on the development of keratitis. Corneal opacification was monitored daily by slit-lamp examination, and clinical scores were graded by the intensity and extent of corneal opacity. As shown in Fig. 2, C57Bl/6 mice injected intraperitoneally with control rat IgG developed pronounced corneal opacification, which persisted throughout the course of the study. In contrast, while mice receiving GK1.5 developed corneal opacification on day 1, this resolved completely by day 3, and corneas were transparent throughout the remainder of the study. These observations demonstrate an essential role for CD4\(^+\) T cells in later-stage corneal pathology.

Previous work demonstrated that the development of *O. volvulus*-mediated keratitis is associated with biphasic recruitment to the corneal stroma, with neutrophils prominent in the first 3 days and eosinophils comprising the majority of inflammatory cells after this time (3, 9). To determine if the...
absence of disease in CD4-depleted mice is due to impaired migration of neutrophils and eosinophils. Immunized mice were sacrificed 1 day or 7 days after intrastromal challenge, and eyes were fixed in formalin and embedded in paraffin by standard methods. To detect neutrophils, 5-μm sections were immunostained with anti-neutrophil monoclonal antibody (NIMP-R14) followed by biotinylated rabbit anti-rat Ig (BioGenex, San Ramon, Calif.). Eosinophils were detected using rabbit antisera to eosinophil major basic protein (1:5,000) and goat anti-rabbit Ig as a secondary antibody (Dako, Carpenteria, Calif.). Cells were visualized using Vector Red Substrate (Vector Laboratories, Burlingame, Calif.) containing Levamisole (Sigma, St. Louis, Mo.) and were enumerated by light microscopy as previously described (2). As shown in Fig. 3, neutrophil numbers were elevated in the corneas of mice in both groups on day 1 but not on day 7 after intrastromal injection. CD4 depletion had no significant effect on neutrophil numbers in the cornea at either time point. In addition, the distribution of neutrophils throughout the cornea was similar in both groups of mice (day 1: 53.5 versus 55.4% in peripheral cornea, 33.3 versus 28.2% in paracentral cornea, and 13 versus 16.5% in central cornea).

In marked contrast to the effect on neutrophils, depletion of CD4+ cells significantly impaired eosinophil recruitment to the stroma following intrastromal challenge with O. volvulus antigens (Fig. 4). This difference was apparent on both day 1 (84.1% reduction) and day 7 (91.4% reduction) after intrastromal injection. As noted above, CD4 depletion did not prevent the development of opacification on day 1, despite the reduction of eosinophils at that time point. However, opacification was significantly abrogated by day 7 compared with control mice. Together, these findings indicate that later-stage O. volvulus keratitis correlates with recruitment of eosinophils into the corneal stroma, whereas keratitis on day 1 is likely due to neutrophils.

As antibody is essential for the development of keratitis (2), we determined if the effects of CD4 depletion were due to altered antibody responses. Sera were assayed for antibody by enzyme-linked immunosorbent assay using biotinylated goat anti-mouse isotype-specific antibodies (Southern Biotechnology, Birmingham, Ala.) as described previously (2). As shown in Fig. 5, O. volvulus-specific IgG1 and IgG2a titers were similar for both groups of mice. These data are also consistent with the notion that the Th2 profile was not affected significantly by CD4 depletion. Blood eosinophils (enumerated by differential
stain of blood smears [Diff-quik; Dade Diagnostics, Aguada, P. R.] were not reduced in CD4-depleted animals; rather, they were significantly elevated (85 ± 51 eosinophils/mm³ versus 260 ± 86 eosinophils/mm³; P = 0.0045). This may reflect the failure of eosinophils to migrate into the tissues.

Although the mechanism underlying CD4-dependent migration of eosinophils to the cornea in O. volvulus keratitis has yet to be determined, at least two possible explanations can be envisioned: (i) production of chemotactic cytokines that directly recruit eosinophils to the cornea, and (ii) production of regulatory cytokines that elevate expression of adhesion molecules on vascular endothelial cells on limbal vessels. T cells produce several chemokines with reactivity for eosinophils, including eotaxin, MIP-1α, and RANTES (4). It has been demonstrated that these chemokines are up-regulated in corneas of mice with exacerbated disease (11). Consistent with this finding, eosinophil migration into the corneas of Scotoxan gene knockout mice is significantly impaired after intrastromal injection of parasite antigens, indicating that eotaxin contributes to inflammatory cell recruitment in this model (13). Studies are under way to determine if CD4 cells are the source of eotaxin and other chemokines in O. volvulus keratitis.

CD4+ T cells may also regulate inflammatory cell recruitment by secretion of cytokines that modulate vascular adhesion molecule expression. For example, IL-4 up-regulates P-selectin (15) and Vascular cell adhesion molecule 1 (VCAM-1) (6, 7), which contribute to eosinophil recruitment without affecting neutrophil migration (8, 14). This is consistent with the previous finding that IL-4 is required for the development of O. volvulus-induced keratitis (10) and that eosinophil recruitment to the cornea is impaired in mice deficient in P-selectin (3a).

CD4+ T cells are present in the cornea in O. volvulus keratitis (1, 10), and it is likely that eosinophil migration is regulated by cells at this site. Further studies using immunolocalization techniques will determine if CD4+ T cells in the cornea are the source of chemokines and immunoregulatory cytokines.

In summary, in addition to the previously described role for CD4+ T cells in induction of a Th2 response, the current findings indicate that CD4+ T cells contribute to keratitis by regulating eosinophil recruitment to the cornea. These observations also appear to define the relative contribution of neutrophils and eosinophils to O. volvulus keratitis, with neutrophils associated with the early phase of corneal disease and with eosinophils required for the development of sustained opacification. Future studies will determine the molecular basis for these observations.

We thank Achim Hoerauf (Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany) for monoclonal antibody NIMP-R14. This work was supported by National Institutes of Health grants EY10320 (E.P.) and FY06913 (L.R.H.) and Burroughs Wellcome New Investigator Award 0720 (E.P.). Funding was also provided by NIH grant EY11373, by the Ohio Lions Research Foundation, and by the Research to Prevent Blindness Foundation. J.T.K. is a recipient of a scholarship award from the German National Merit Foundation, Stu- dienstiftung des deutschen Volkes.

REFERENCES


Editor: W. A. Petri, Jr.