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Immunopathology of ocular onchocerciasis. I. Inflammatory cells infilitrating the anterior segment

C. C. CHAN,* E. A. OTTESEN,† K. AWADZI,‡ R. BADU‡ & R. B. NUSSENBLATT* * *National Eye Institute; and † National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA; and ‡ Onchocerciasis Chemotherapeutic Research Centre, Tamale, Ghana*

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SUMMARY

Ocular tissue (conjunctiva and iris) was obtained from 12 adult African men with active ocular onchocerciasis and from nine age-matched persons from the same endemic region but without onchocercal infection. These tissues were examined immunohistologically and two major findings were noted. First, mild-to-moderate chronic inflammatory cellular infiltration was present in the conjunctiva of the onchocerciasis patients. T lymphocytes ($CD3^+$) were the major inflammatory cells, and the T suppressor/cytotoxic ($CD8^+$) subset was significantly increased in the ocular onchocerciasis patients ($P < 0.03$). Second, in the onchocerciasis patients, non-lymphoid cells of the conjunctiva and iris, such as vascular endothelium, pericytes and fibroblasts were in an activated state, as shown by increased expression of Class II MHC antigens ($P < 0.02$, conjunctiva; $P < 0.05$, iris). These concomitant findings of lymphocyte infiltration and resident cell activation indicate a dynamic state of localized host responsiveness presumably to the microfilarial parasites and their products in the anterior segments of the eyes of patients with ocular onchocerciasis.

Keywords onchocerciasis conjunctiva iris immunopathology ocular inflammation

INTRODUCTION

Onchocerciasis, the second most common infectious cause of blindness, occurs in an estimated 20 million people in endemic areas of Africa, Central and South America and causes blindness in about one million (Thylefors, 1978; WHO Expert Committee, 1987). It is the consequence of infection by the tissue-dwelling parasitic nematode *Onchocerca volvulus* that is transmitted by black flies of a number of Simulium species. The predominant ocular manifestations of onchocerciasis are limbitis, superficial punctate and sclerosing keratitis, anterior and posterior uveitis, chorioretinal degeneration and optic atrophy (Thylefors 1978; Bell, 1985). Secondary effects such as cataract and glaucoma may also occur (Thylefors, 1978; Bell, 1985). As whole villages have been known to have large proportions of their adult populations rendered blind as a result of this disease, onchocerciasis has been recognized by the World Health Organization (WHO) as one of the five major preventable blinding conditions (WHO Expert Committee, 1987).

Despite the great numbers of affected people, our understanding of the pathology and pathogenesis of ocular onchocerciasis has been severely limited by the scarcity of eye tissue available for examination; and the examined case material so far has represented mostly the advanced stages of the eye disease (Rodger, 1960; Paul & Zimmerman, 1970; Garner, 1976). Furthermore, there have been no studies to evaluate systematically with immunohistological techniques the local cellular immune alterations in either acute or chronic onchocercal eye disease.

We therefore took advantage of the opportunity to collect biopsy specimens of conjunctiva and iris tissue at the time of cataract surgery in 12 patients with chronic, active ocular onchocerciasis and from nine individuals, living in the same endemic area, with cataracts but without onchocercal infection; our hope was that detailed histologic and immunohistologic comparisons may provide new information on immunologic mechanisms likely to be involved in the pathogenesis of this disease. Our findings indicate that the anterior segment tissues in chronic ocular onchocerciasis have two characteristic features that distinguish them from normal eye tissues; first, they are infiltrated with chronic inflammatory cells that are predominantly lymphocytes expressing the CD8 marker, and, second, there is a marked activation of resident cell populations (vascular endothelium, pericytes and fibroblasts), as determined by the expression of major histocompatibility class II antigens.

Correspondence: Chi-Chao Chan, M.D., National Eye Institute, Building 10, Room 10N206, National Institutes of Health, Bethesda, MD 20892, USA.

MATERIALS AND METHODS

Patient population

Twenty-one men from the area of Tamale in northern Ghana

Table 1. Clinical findings of Ghanan patients with and without onchocerciasis

	Onchocerciasis patients	Non-onchocerciasis patients
Age* (Years)	59 (49–65)	55 (37–70)
Skin:		
Itch	6/12	3/9
Pigment Change	11/12	3/9
Nodules	12/12	0/9
Microfilariae/mg*	6·5 (1·6–57)	0
Eye:		
Itch	7/12	3/9
Visual Acuity (6/60 or better)	7/12	5/9
Sclerosing Keratitis	9/12	0/9
Fluffy Opacities	7/12	0/9
Microfilariae in		
Cornea (live)*	4/12 [2·5(1–4)]	0/9
Cornea (dead only)*	6/12 [2·5(1–49)]	0/9
Anterior Chamber (live)*	11/12 [12(1–>50)]	0/9
Uveitis	12/12	0/9
Cataract(s)	12/12	9/9
Posterior Changes		
Not Evluatable	8/12	7/9
Present	4/4	0/2

* Median (and range).

were given thorough medical evaluations by history, clinical laboratory, physical, and ocular examinations carried out according to a standardized WHO-recommended protocol (WHO Expert Committee, 1976). As seen in Table 1, all had cataracts in one or both eyes, but only 12 had evidence of onchocerciasis either ocular or systemic. All of these 12 had microfilariae in the skin—evaluated by skin snips made with a 2·0-mm Walzer-type corneoscleral punch at the left outer canthus, right and left scapulas, right and left iliac crests and right and left calves (Francis, Awadzi & Ottesen, 1985)—and in the eye (identified by slit lamp), and all had palpable subcutaneous onchocercal nodules. The remaining nine individuals were comparable to the infected patients in age and general health but had no clinical or clinical laboratory evidence defining onchocercal infection. While it is possible that some of these nine may have had onchocerciasis in the past, at the time of this study there was no way to document their past histories more precisely. None of the onchocercal or non-onchocercal patients was receiving medication for onchocerciasis or other conditions, and none was known to have been treated for onchocerciasis previously. While the conjunctiva was similar and grossly unremarkable in both groups of patients, posterior segment changes were seen in the four patients whose retinas could be visualized prior to surgery but not in the two evaluatable non-onchocercal patients. Detailed posterior segment evaluations were not performed after surgery.

Surgical procedure

All patients underwent clinically indicated, uncomplicated intracapsular cataract extractions. During the surgical procedure, a small piece ($\sim 3 \times 5$ mm^2) of conjunctiva was obtained from the superior bulbar region. A peripheral iridectomy was also performed on all patients, and the approximately 2×1 mm^2 iris specimen was saved for study. Conjunctiva and iris specimens were immediately embedded in OCT (optimal cutting temperature compound, Miles Laboratory) and frozen in liquid nitrogen. The clinical protocol for these studies was approved by both the Ghanian Ministry of Health and the U.S. National Institutes of Health, and informed consent was obtained from each patient prior to the study.

Immunohistologic techniques

Frozen sections of the conjunctiva and iris specimens were stained by an avidin–biotin–immunoperoxidase technique described in detail previously (Hsu, Raine & Fanger, 1981; Chan *et al.*, 1986a) with monoclonal antibodies directed against subsets of inflammatory cells and major histocompatibility complex (MHC) class II antigens. Monoclonal antibodies used (Becton-Dickinson, CA and Ortho Laboratory, NJ) identified the following: a *pan* T lymphocyte, CD3, marker (Leu 4); the T-helper/inducer, CD4, subset (Leu 3a); the T suppressor/cytotoxic, CD8, subset (Leu 2a); B, CD22, lymphocytes (Leu 14); macrophage/monocyte, CD11, marker (OKM1); NK, CD16, cells (Leu 7); MHC class II-DR (HLA-DR) and MHC class II-DQ antigens (Leu 10). The secondary antibody was biotinylated horse anti-mouse IgG (Vector Laboratory). The substrate was 3-3′ diaminobenzidine.

The number of cells staining positive for each cellular marker in each specimen was calculated $\times 400$ power microscopy. Total numbers of cells in each high-power field (HPF) of the entire specimen (15 sections/specimen) were counted and averaged. Mild, moderate and severe cellular infiltration in the specimen were arbitrarily defined as < 10 cells/HPF; 10–50 cells/HPF; and > 50 cells/HPF, respectively.

Statistical analysis

Student's *t*-test comparing geometric means and Fisher's exact test comparing frequencies were used to evaluate differences between the non-onchocercal individuals and the ocular onchocerciasis patients.

RESULTS

Infiltrating inflammatory cells

Conjunctiva. All individuals studied, both with and without onchocerciasis, showed at least mild cellular infiltration in the substantia propria. The infiltrating cells were predominantly mononuclear cells which immunohistologically proved to be $> 80\%$ T cells (Fig. 1). The most heavily infiltrated specimens came from the ocular onchocerciasis group, with seven of the nine individuals who had more than 15 T cells/HPF in that group (Fig. 2a).

Analysis of the subsets of these infiltrating T cells showed that there were significantly greater numbers of CD8+ (suppressor/cytotoxic phenotype) T cells in the patients with ocular onchocerciasis than in the non-onchocercal population ($P < 0{\cdot}03$). There were trends toward both greater total T (CD3+) and helper-phenotype (CD4+) T cell infiltrations in the conjunctivae of the onchocerciasis patients (Fig. 2a), but the differences between the groups did not reach statistical significance.

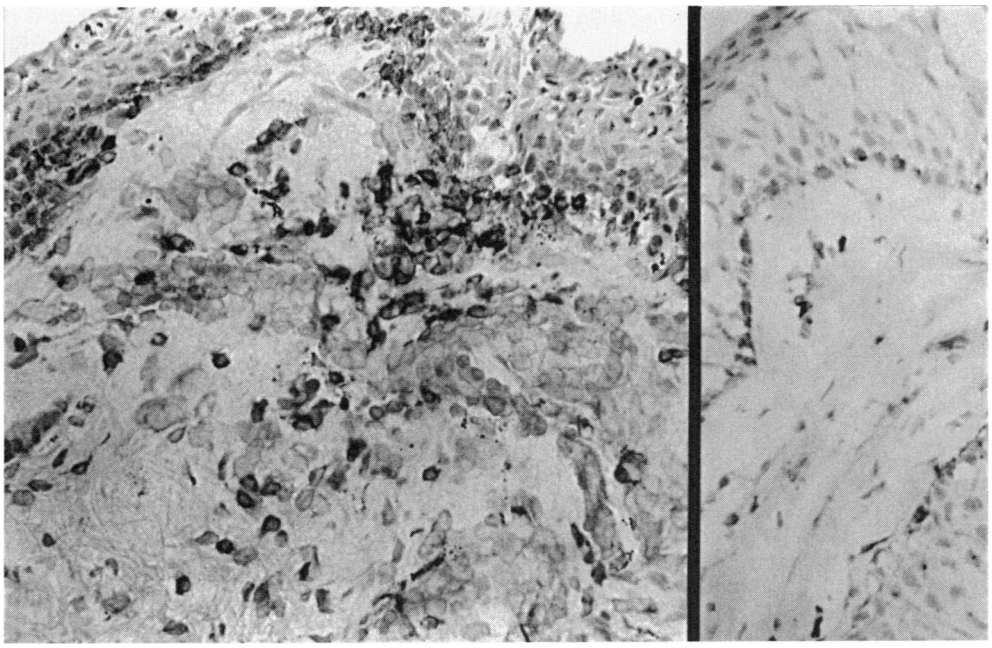

Fig. 1. The majority of cells infiltrating the conjunctiva of a patient with onchocerciasis stained positively for CD3 (left) (avidin–biotin–immunoperoxidase, ×250); and the occasional inflammatory cells infiltrating the conjunctiva of a patient without onchocerciasis stained positively for CD3 (right) (avidin–biotin–immunoperoxidase, ×250).

Small numbers of other cell types were found infiltrating the conjunctiva in both groups. Eosinophils were rarely seen; B cells (CD22+), natural killer (NK) cells (CD16+), and macrophages (CD11+) were present in similar numbers in both onchocercal and non-onchocercal patients and thus did not relate specifically to onchocercal infection.

When the character of the cellular infiltrations was evaluated for correlations with the various clinical manifestations of onchocercal ocular disease identified (Table 1), the three infected patients who were free of sclerosing keratitis were found to have significantly greater numbers of total T cells (CD3+), B cells (CD22+) and monocytes (CD11+) in their conjunctival biopsies than did the nine patients with sclerosing keratitis ($P<0.05$ for each comparison; Fig. 2b). No other clinical/pathological relationships were noted.

Iris. In the iris specimens from all individuals studied, few T lymphocytes or other inflammatory infiltrating cells were seen. Indeed, in about half of the individuals from each group no infiltrating cells could be seen, and no significant differences were noted between these two groups.

Non-lymphoid resident cells expressing MHC class II antigens

Conjunctiva. When activation markers on the resident, non-lymphoid cells of the conjunctiva (vascular endothelium, pericytes and fibroblasts) were evaluated by staining for HLA-DR and HLA-DQ markers, the patients with onchocercal ocular disease were found to have a significantly greater percentage of their resident cells activated than did the non-infected, control population ($88.3\% \pm 5.9$ *versus* $59.3\% \pm 9.5$; $P<0.02$; Fig. 3).

Iris. Accurate quantification of all activated non-lymphoid, resident cells of the iris specimens was not possible because of the presence of the dense iris melanin pigment. However, the presence or absence of the HLA-DR and HLA-DQ activation markers on vascular endothelial cells could be assessed qualitatively (1+ to 4+). Such activation was found in all of the onchocerciasis patients and in five of the eight non-onchocercal patients studied, and was qualitatively more prominent in the onchocerciasis patient group.

DISCUSSION

Despite the importance of onchocerciasis as a cause of blindness in Africa and South America, our understanding of the mechanisms underlying its pathogenesis is rudimentary (Taylor, 1985). Although attempts are being made, the establishment of animal models for onchocercal eye disease has been particularly difficult both because successful infection with *Onchocerca volvulus* parasites appears restricted to humans, gorillas and chimpanzees and because so little ocular tissue from patients with ocular onchocerciasis has been available for examination (particularly with immunological techniques) that even the validity or relevance of observations made in animal models has been difficult to assess. Thus, it is particularly important that tissue from affected human eyes be obtained and evaluated thoroughly.

In the present study, conjunctiva and iris specimens removed from the eyes of 12 onchocerciasis patients undergoing cataract surgery were studied histologically and immunohistologically. There were no abnormalities seen grossly in these specimens and no parasites identified, but four major findings were noted: (i) conjunctival specimens showed chronic inflammatory cell (primarily T lymphocyte) infiltration; (ii) suppressor/cytotoxic ($CD8^+$) phenotype T cells predominated in these infiltrations; (iii) the character of the inflammatory infiltrate differed between the patients with sclerosing keratitis and those without this complication; and (iv) the non-immune resident cell populations

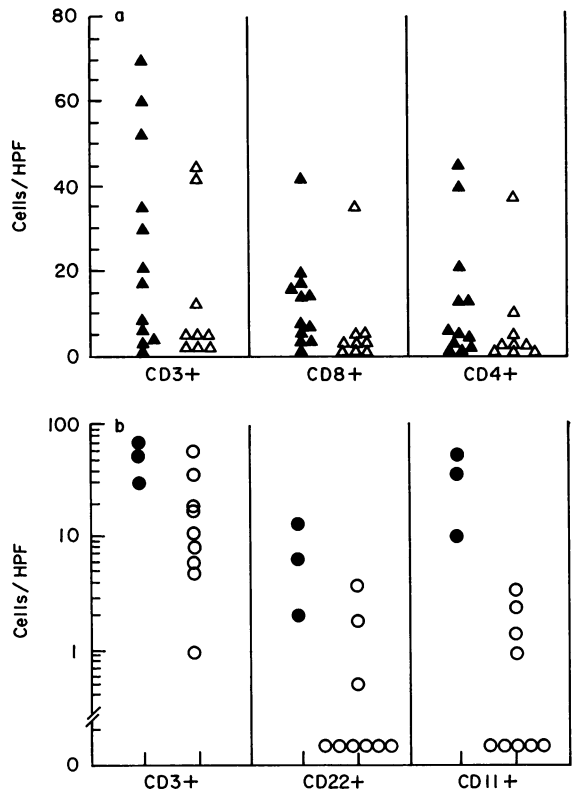

Fig. 2. (a) Mean numbers of conjunctival T ($CD3^+$) lymphocytes and the $CD8^+$ and $CD4^+$ T-cell subsets per ×400 high-power field (HPF). (Left-column symbols indicate the study patients with onchocerciasis; right-column symbols indicate the study subjects without onchocerciasis). (b) Mean numbers of conjunctiva-infiltrating mononuclear cells (CD3+ T cells, CD22+ B cells and CD11+ monocytes/macrophages) per ×400 high-power field for ocular onchocerciasis patients either with (right-column symbols) or without (left-column symbols) sclerosing keratitis.

(vascular endothelium, pericytes and fibroblasts) of both the conjunctiva and iris were in an 'activated' state, as judged by the presence of the class II MHC antigen surface markers.

Few lymphocytes have been reported in conjunctival specimens from normal individuals living in North America or China (Bhan, Fujikawa & Foster, 1982; Chan *et al.* 1988) in contrast to our findings in the conjunctival biopsies from the nine African patients with cataracts but without onchocerciasis in the present study. These latter specimens showed moderate T cell infiltration that presumably reflects greater exposure to ocular pathogens or irritants for the general population of rural Ghana. It is also possible that some of these uninfected 'controls' had had previous onchocercal infection involving the eye, but at the time of evaluation there was no clinical, laboratory or parasitologic evidence for such earlier infection. Indeed, the prominent T-lymphocyte infiltrations seen in two of the non-onchocerciasis patients (one in whom $CD8^+$ and the other, in whom $CD4^+$ predominated; Fig. 2a) remain unexplained but do suggest that the non-onchocercal patients in this study were not an homogeneous group of 'normal' individuals.

Despite this 'high background' of conjunctival lymphocyte infiltration, the onchocerciasis patient population did show distinctly greater numbers of infiltrating T cells (especially $CD8^+$ cells) than did the non-onchocercal patients. In other ocular diseases, including vernal conjunctivitis, pemphigoid, Mooren's ulcer and sarcoidosis (Bhan *et al.*, 1982; Semenzato *et al.*, 1986) a predominantly T cell infiltration of the conjunctiva has also been reported. T cell-mediated immune responsiveness is thought to play an important role in pathogenesis of these disorders, and it is likely that the marked T cell infiltration seen in the ocular specimens from onchocerciasis patients in the present study has similar implications.

Predominance of the suppressor/cytotoxic ($CD8^+$) phenotype in the T cells infiltrating conjunctival tissue has been seen previously in a number of conditions where chronic ocular inflammation is a consistent feature, e.g. trachoma (Whittum-Hudson, Prendergast & Taylor, 1986). Thus, this finding in patients with onchocerciasis may reflect some general, though still undefined, role of $CD8^+$ cells in chronic ocular inflammatory disorders. Alternatively, since immunologic suppressor mechanisms are prominent features of the host response to *O. volvulus* (Bartlett *et al.*, 1978; Donnelly *et al.*, 1984) and other filarial (Ottesen, 1984) and helminthic infections (Nussenzweig, 1982), the $CD8^+$ infiltrates in the conjunctivae of these patients might be the local manifestation of general, and more specifically parasite-oriented host immune responses. The surprising finding of significantly greater numbers of infiltrating T cells, B cells and macrophages in the conjunctivae of pateints without sclerosing keratitis could also be important. While the presence of these cells might just reflect early stages of corneal pathology, it is also possible that these inflammatory cells play a defensive role in the prevention of corneal inflammation and scarring induced by ocular microfilariae.

The absence of eosinophils from these specimens was surprising in view of the prominence of these cells in the acute ocular inflammation induced by treatment of patients with ocular onchocerciasis (Mackenzie, 1980). Perhaps the chronic nature of the inflammatory response in our patients accounts for the absence of eosinophils; it would be interesting to stain these tissues for major basic protein, one of the degranulation products of eosinophils that has been shown to persist in tissues long after acute eosinophil-induced inflammation has subsided (Lieferman *et al.*, 1985).

Of particular note, however, was the finding that non-lymphoid resident cells in the eyes of patients with onchocerciasis abundantly expressed MHC class II antigens. In normal conjunctivae, only a few Langerhans and vascular endothelial cells express such antigens (Bhan *et al.*, 1982; Chan *et al.*, 1988b). Enhancement of MHC class II antigens on the resident cells in the iris has been found in various ocular diseases, particularly associated with uveitis (Stevens *et al.*, 1987; Ni *et al.*, 1988). Whether this cellular activation seen in our patients with ocular onchocerciasis is immune mediated, e.g. by interferon-gamma released by the infiltrating T cells as described in other conditions (Young, Stark & Pendergast, 1985; Chan *et al.*, 1986a; Fujikawa *et al.*, 1987) or whether it is parasite mediated, e.g. by some soluble factor released from the microfilariae themselves, is uncertain. Interestingly, other parasites have been recently shown to elaborate such cellular activation factors (Freedman & Ottesen, 1988).

To place the findings from this study in the context of the progressive development of ocular disease during onchocercal infection, it is important to recognize that all 12 study patients had lived for decades in a region of heavy *O. volvulus*

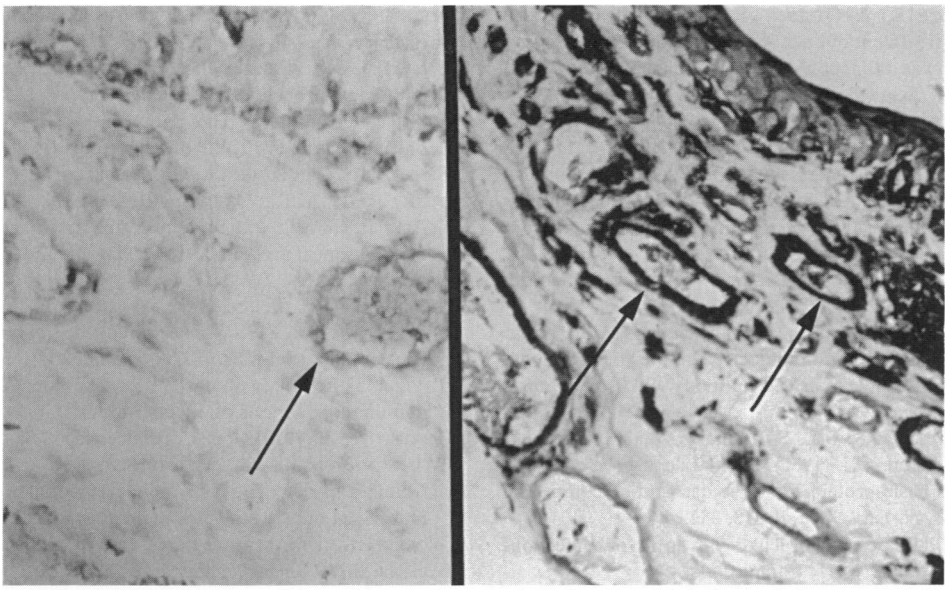

Fig. 3. Vascular endothelial cells (arrow) in the conjunctiva of a study subject not having onchocerciasis, unstained by reagents to detect HLA-DR (left); the pale staining results from the methyl-green counter stain; and vascular endothelial cells (arrows) stained positively after similar treatment from the conjunctiva of a patient with ocular onchocerciasis (right) (avidin–biotin–immunoperoxidase, × 250).

transmission and it is likely that they had had onchocerciasis for at least several decades. Ongoing transmission of the parasite, however, had been severely reduced or halted in this region by vector control efforts of the Onchocerciasis Control Programme (Bell, 1985; Taylor, 1985) during most of the previous 5–7 years. Though new infections were unlikely during this period, the life span of *O. volvulus* probably exceeds one decade (WHO Expert Committee, 1987), so that it is not surprising that 11 of the 12 patients still demonstrated live microfilariae in the anterior chamber and all had living parasites in the skin snips. Thus, the immunopathologic changes observed in the eyes of these patients must reflect the pathologic features of chronic, not acute or early onchocercal eye disease.

However, these patients were also not those with the most severe manifestations of ocular onchocerciasis, since the most severely affected of their community likely developed blinding onchocerciasis many years earlier at a younger age. The patients in the present study were old and, in the few whose posterior segments could be evaluated before surgery, showed at least some abnormality there; still, it was felt clinically that these individuals had enough visual potential to warrant surgery for removal of their cataracts which had developed probably incidentally to their infections with *O. volvulus* (WHO Expert Committee, 1987). Indeed, these individuals appeared to have come to some type of host-parasite (immunologic ?) 'compromise' with their infections that resulted in persistence of the parasite and some measure of ocular inflammatory pathology but not such severe inflammatory changes that visual impairment was complete. Finally, it is also likely that not all 12 of the onchocerciasis study patients were at the same 'stage' of their disease. Nine of the 12 had the characteristic presentation of sclerosing keratitis, but three did not, and, probably significantly, the character of the immunologic inflammatory response in the conjunctivae of these three individuals differed from that of the other nine.

Although still a long way from defining the immunopathogenesis (or even the basic immunopathology) of ocular onchocerciasis, the present findings emphasize the potential importance of T cell-mediated immune processes in the anterior segment lesions. It is important that such findings be extended with more immunologic and immunohistologic observations of the ocular lesions of patients with onchocercal infection, not only to increase our general understanding of this blinding disorder but also so that the criteria necessary for developing appropriate, more easily studied animal models for this infection can be defined.

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