Immunologic Cross-Reactivity in the Pathogenesis of Ocular Onchocerciasis

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Purpose. *Onchocerca volvulus,* a filarial worm, is a major cause of infectious blindness and inflammatory eye disease. An autoimmune cause for ocular onchocerciasis has been suggested since the identification of a recombinant antigen of *O. volvulus* that shows immunologic cross-reactivity with a host ocular component of 44,000 M_r. The aim of this study was to establish the distribution of the cross-reactive antigens in both host tissues and the parasite, and to determine if significant autoantibody responses to the host antigen could be detected in infected persons.

Methods. The tissue and organ distribution of the 44,000 M_r antigen was determined by immunocytochemistry and Western blot analysis. Human autoantibody responses to the ocular antigen were demonstrated by Western blot analysis using sera collected from persons with onchocerciasis, with and without posterior segment pathology, Bancroftian filariasis, and Europeans with no filarial infection.

Results. The tissue distribution of the 44,000 M_r antigen correlates with the sites of pathology in onchocerciasis and antibody reactivity against this antigen could be detected in all persons with onchocerciasis and posterior segment pathology. The antigen is also recognized by sera from persons with Bancroftian filariasis, but not from normal persons.

Conclusions. A role is proposed for immunologic cross-reactivity in the pathogenesis of onchocerciasis and it is suggested that intraocular presentation of the cross-reactive parasite antigen by microfilariae is essential for the development of disease. Invest Ophthalmol Vis Sci. 1993;34:2888-2902.

*Onchocerca volvulus* is a filarial nematode and the causative agent of onchocerciasis (river blindness). Onchocerciasis is a major blinding disease in Africa, Central America, and South America. An estimated 85 million people live in endemic areas. Approximately 18 million people are infected and about 350,000 people are blind as a result of the disease.1

Ocular disease has been divided into anterior and posterior segment onchocerciasis. Anterior segment disease is characterized by punctate keratitis, sclerosing keratitis, and iridocyclitis. Posterior segment disease includes optic neuritis, optic atrophy, and chorioretinitis.2-5

*Onchocerca* is probably the largest single cause of uveitis and represents a unique opportunity to investigate inflammatory disease of the eye. The mechanism by which infection with *O. volvulus* leads to ocular disease remains obscure. Inflammatory responses directed against dead microfilariae may explain the development of pathologic changes in the anterior segment of the eye.6,7 The pathogenesis of the posterior segment disease has been unclear. Migrating microfilariae,8,9 circulating immune complexes,10 and direct interaction of cells of the immune system with microfilariae have been implicated.11 Regarding an autoimmune cause there has been considerable interest in the role of humoral and cellular responses to retinal antigens, principally S-antigen and interphotoreceptor retinoid binding protein (IRBP).12-14 However, antibody and T-cell responses to either of these
proteins do not correlate with posterior segment disease. Peptides derived from viral and bacterial proteins that show sequence homology with the uveitogenic portion of S-antigen are capable of inducing experimental autoimmune uveitis in susceptible rats.15 Neither S-antigen or IRBP have been reported to show immunologic cross-reactivity with O. volvulus.

Molecular mimicry has been suggested as a mechanism for the induction of autoimmune disease after viral or bacterial infections.16 It has been hypothesized that foreign antigens with epitopes similar to a host determinant may, under some circumstances, induce autoimmune disease. Recently, using pooled human infection sera to screen a λgt11 library of adult female O. volvulus we identified a recombinant antigen of O. volvulus that was shown to be immunologically cross-reactive with a 44,000 M₃ component of the retinal pigment epithelium (RPE).17 The current investigation was conducted to determine if the 44,000 M₃ antigen has a role in the pathogenesis of ocular onchocerciasis.

MATERIALS AND METHODS

Preparation of DNA

Genomic DNA from O. volvulus, Brugia malayi, Acanthocheilonema vitae, and human DNA from a hybrid cell line ESH98,18 was isolated from homogenized material treated with proteinase K in sarcosyl/ethylenediaminetetraacetic acid, as described by Maniatis et al.19 Genomic DNA of O. gibsoni, O. gutturosa, O. armillata, and B. pahangi was donated by Prof. R. Post, University of Wageningen, Holland.

Hybridization Reactions

DNA/DNA hybridizations were performed under high stringency conditions in 50% formamide at 42°C using the radiolabeled complementary DNA insert as a probe. This probe, prepared by the random primer labeling technique,20 was used for hybridization21 of Southern blots.22

Sources of Sera

Onchocerciasis infection sera was collected from patients in Lunsar, Sierra Leone. The ocular disease status of the patients was determined after an ocular examination conducted by Clare Gilbert. These patients were divided into two groups: those with and without posterior segment pathology (16 and 11 patients, respectively). Sera from individuals with Bancroftian filariasis, and sera from healthy Europeans with no history of filarial infection or uveitis were used as controls. The sera from 12 patients with Bancroftian filariasis were donated by Professor R. Sripathy Prasad (University of Kerala, Kariavattom, Thiruvananthapuram, India).

Rabbit sera were taken before and after immunization with purified or semipurified fusion proteins of Ov39 expressed in pEX34b (designated EX39) or pGEX1 (designated GEX39) and the electroeluted 44,000 M₃, ocular antigen. Clone Ov39 is a recombinant antigen of O. volvulus that has been described previously.17 Rabbit sera raised to the EX39 fusion protein was adsorbed as follows: 30 ml of sera was adsorbed against 1 ml of ×100 concentrated bacterial lysate containing the carrier protein or the EX39 fusion protein.

Disease Status of Onchocerciasis Patients

All onchocerciasis patients included in the study were skin-snip positive for microfilariae. The clinical findings are summarized in Table 1.

Anterior segment examinations were performed on a Haag-Streit (Koniz, Switzerland) slit lamp. The presence of microfilariae in the anterior chamber was determined after the patient had been put in a head-down position for 2 minutes. Eye examination followed immediately using ×16 magnification in the eyepiece.

Posterior segment examinations were performed using direct and indirect ophthalmoscopy after dilating the pupils with 1% cyclopentolate and 10% phenylephrine. Some examinations were also made on the slit lamp with a 90 diopter lens. The definitions used in the assessment of disease were as follows:

- Sclerosing keratitis: peripheral corneal scarring with vascularization.
- Active uveitis: presence of cells and flare in the anterior chamber with or without posterior synechiae.
- Inactive uveitis: posterior synechia or typical onchocerciasis down drawn miosed pupil without cells and flare.
- Chorioretinitis: RPE thinning and atrophy, and chorioretinal atrophy, with or without subretinal scarring, were all classified as positive.
- Optic atrophy: nonglaucomatous pallor of the disc together with a positive skin snip was taken as indicative of onchocercal optic atrophy.
- Glaucoma: intraocular pressure measurements were taken using a Goldman application tonometer (Haag-Streit). Glaucoma was defined as an intraocular pressure greater than 25 mm Hg in the presence of optic disc cupping, or an intraocular pressure of greater than 40 mm Hg without glaucomatous optic disc cupping.

Preparation of Antigens for Western Blotting

Bovine eyes were obtained from a local abattoir and transported to the laboratory on ice for immediate...
Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis and Western Blot Analysis

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed as described by Laemmli.23 Electrophoretically separated polypeptides were transferred to nitrocellulose paper (BA 85, Schleicher and Schuell (Dassel, Germany)) for 2 hours at 400 mA, following the method of Towbin et al.25 For immunostaining, nonspecific sites were blocked with 3% bovine serum albumin, 5% goat serum in Tris-buffered saline (50 μmol/l Tris-HCl pH 7.4, 150 μmol/l NaCl). The primary antibody was used at a dilution of 1:300 (rabbit serum) or 1:100 (human serum and adsorbed rabbit serum) in Tris-buffered saline containing 1% bovine serum albumin, 0.05% sodium azide. For the demonstration of the cross-reactivity of sera raised to the 44,000 Mr ocular antigen with microfilariae, the sera were used at a dilution of 1:80. Western blots were incubated overnight in the appropriate sera. After three changes of Tris-buffered saline the binding of the primary antibody was detected with goat anti-rabbit or goat anti-human peroxidase conjugates (Sigma Chemical Co., Dorset, UK), used at 1:1000 dilution in Tris-buffered saline containing 1% bovine serum albumin, 0.05% sodium azide. For the demonstration of the cross-reactivity of sera raised to the 44,000 Mr, ocular antigen with microfilariae, the sera was used at a dilution of 1:80. Western blots were incubated overnight in the appropriate sera. After three changes of Tris-buffered saline the binding of the primary antibody was detected with goat anti-rabbit or goat anti-human peroxidase conjugates (Sigma Chemical Co., Dorset, UK), used at 1:1000 dilution in Tris-buffered saline containing 1% bovine serum albumin. After three more washes in Tris-buffered saline and a final wash in PBS, binding of the peroxidase conjugate was demonstrated using 4-chloro-1-naphthol and H2O2 as described previously.17

Purification of the 44,000 M, Antigen from Optic Nerve

Bovine optic nerve extracts were prepared for sodium dodecyl sulfate-polyacrylamide gel electrophoresis as

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**TABLE 1. Clinical Findings in the Onchocerciasis Patients**

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Sera from these patients were used in the Western blot experiments shown in Figures 9, 10C, and 10D. The abbreviations used are as follows: Pat No, patient number; y, yes; n, no; m, male; f, female; MAC, microfilariae in anterior chamber; SK, sclerosing keratitis; Uv, uveitis (a, active or ia, inactive); Cat, cataract; Gl, glaucoma; Ch, chorioretinitis; OA, optic atrophy.
Cross-Reactive Antigens and Ocular Onchocerciasis

Preparation of Tissues for Immunocytochemistry

Cryostat sections of bovine neuroretina and RPE were prepared from fresh bovine eyes. Sections of retina were immersed in PBS to remove embedding media. Alternatively, sections were fixed in 10% formal saline and processed conventionally for wax histology. Histologic sections of a human eye were incubated with the primary antibody for 2 hours. After several washes in PBS, binding of immunoglobulins was blocked by a 20-minute incubation in 1% goat serum. Primary antibodies were used at a range of dilutions between 1:10 and 1:200. Sections were incubated with the primary antibody for 2 hours. After further washes in PBS, binding of immunoglobulins was detected by a 1-hour incubation with goat anti-rabbit peroxidase conjugate or goat anti-rabbit immunoglobulin. Controls for immunocytochemical specificity were: (1) omission of the primary antibody; (2) the use of sera obtained from the rabbit before immunization at the same dilution as the test sera; and (3) the use of sera raised to the carrier peptides of the fusion proteins.

RESULTS

The recombinant antigen, Ov39, is an antigen of *O. volvulus* that is immunologically cross-reactive with a 44,000 M, component of the RPE.17 Its cloning history and molecular characterization has been described. A recently repeated search of data bases (PIR1, PIR2, PIR3, SWISS-Prot, and NEWAT84) for protein sequence homology did not identify any similarities with cataloged entries.

Species Specificity of Ov39

The species specificity of clone Ov39 was tested by DNA:DNA hybridization of DNA of *Loa loa* and *Acanthocheilonema vitae*. *Brugia malayi*, *Onchocerca gibsoni*, *O. gutturosa*, *O. armillata*, *B. pahangi*. Human DNA was used as a control. Southern blots of *EcoRI* digested DNA derived from these sources were hybridized with the cDNA probe of *O. volvulus*. The corresponding “gene 39” or part of the “gene 39” is located on a 2.3 kb *EcoRI* fragment of genomic DNA of *O. volvulus*. All the other *Onchocerca* species tested have a very closely related gene that can be localized on a 2.1 kb, 2.3 kb and 1.8 kb *EcoRI* fragment of *O. gibsoni*, *O. gutturosa*, and *O. armillata*, respectively. Homologous gene sequences have been identified on an 8 kb fragment of *Loa loa* genomic DNA, and on two fragments of 2.3 kb and 4.2 kb of *B. malayi*. Stringent hybridization conditions reveal only very weak homologies on a 3.7 kb fragment between *B. pahangi* DNA and Ov39, and entirely failed to identify corresponding sequences in the genome of *A. vitae* and human DNA (Fig. 1).

Cross-Reactivity with the 44,000 M, Ocular Antigen of Serum Raised Against EX39

To test the specificity of the cross-reaction, the serum raised to EX39 was adsorbed against a cytosolic extract of bacteria that were induced to over-produce the EX39 fusion protein and to a similar extract containing the expressed carrier protein. Adsorption against the EX39 fusion protein completely removed reactivity to the 44,000 M, antigen in a Triton X-100

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**References:**


Localization of the Cross-Reactive 44,000 Mr Antigen in Ocular Tissues

Immunohistochemical localization of the 44,000 Mr in human, bovine, and murine material was conducted using sera raised to the 44,000 Mr, ocular antigen and the EX39 fusion protein. On cryostat sections of bovine retina the immunofluorescence staining obtained with both sera was markedly enhanced after simultaneous mild detergent extraction and fixation with formal saline (Fig. 5).

The sera raised to EX39 and the cross-reactive antigen failed to detect the ocular component in routinely processed formalin-fixed histopathologic mate-

extract of RPE cells (Figure 2), an extract of optic nerve, and a retinal extract (data not shown). The antibody reactivity was unaffected by adsorption with the carrier protein (Figure 2). Some nonspecific reactivity to irrelevant antigens remained.

Identification of the Native Parasite Antigen

It was previously determined that the native adult worm antigen recognized by the antiserum to the EX39 fusion protein is a 22,000 Mr, PBS-soluble component of adult worms.\textsuperscript{17} Indirect immunoperoxidase staining of nodules containing adult worms and microfilariae (Fig. 3) revealed that the antigen was located in the hypodermis, and the epithelial lining of the uteri of adult female worms. In microfilariae, the antigen was also located internally with prominent staining of the muscle cells. Control sections incubated with sera obtained before immunization and with sera raised to the carrier protein did not stain (Fig. 3). The presence of the antigen in microfilariae was investigated by Western blot analysis. An antigen of approximately 27,000 M\textsubscript{r}, was detected by sera raised to the EX39 and the GEX39 fusion proteins. A similar-sized antigen was weakly recognized by the serum raised to the 44,000 M\textsubscript{r}, ocular antigen (Fig. 4).

\textbf{FIGURE 1.} Autoradiograph of a Southern blot probed with the complementary DNA fragment encoding Ov39. Chromosomal DNA, digested with EcoRI and electrophoretically separated on a 0.7\% agarose gel, was transferred onto nitrocellulose. Panel A: (lane 1) Human chromosomal DNA; (lane 2) O. volvulus DNA; (lane 3) Loa loa DNA; (lane 4) A. vitae DNA; (lane 5) B. malayi DNA. Panel B: (lane 1) O. volvulus DNA; (lane 2) O. gibsoni DNA; (lane 3) O. gutturosa DNA; (lane 4) O. armillata DNA; (lane 5) B. pahangi DNA.

\textbf{FIGURE 2.} Specificity for the 44,000 M\textsubscript{r}, ocular antigen of the serum raised against the recombinant antigen, EX39. Components of the 1\% Triton X-100 fraction of retinal pigment epithelial cells were separated on a 10\% acrylamide/sodium dodecyl sulfate gel under reducing conditions, transferred onto nitrocellulose, and probed with: (lane 1) serum raised to the EX39 fusion protein; (lane 2) serum raised to the EX39 fusion protein adsorbed with bacterial lysate containing the carrier protein; (lane 3) serum raised to the EX39 fusion protein adsorbed with bacterial lysate containing the EX39 fusion protein. Immunoreactivity to the 44,000 M\textsubscript{r} antigen was specifically adsorbed out.
FIGURE 3. Light micrographs illustrating the distribution of the parasite antigen in adult female worm and nodular microfilariae, using rabbit serum raised to the recombinant antigen, EX39. (A) Serum taken from the rabbit before immunization produced only background staining. (B) Serum taken after immunization with the EX39 fusion protein resulted in specific staining of the hypodermis (solid arrow) and epithelial lining of the uteri (open arrow). At higher magnification (insert), staining of the internal structures of microfilariae can be seen. Calibration bar = 200 μm or 20 μm for the insert.
FIGURE 4. Identification of the cross-reactive parasite antigen as a component of microfilariae by Western blot analysis. The components of microfilariae were separated on a 10% acrylamide/sodium dodecyl sulfate gel, transferred onto nitrocellulose, and probed with rabbit serum raised to: (lane 1) the EX39 fusion protein; (lane 2) serum taken before immunization with the EX39 fusion protein; (lane 3) the GEX39 fusion protein; (lane 4) serum taken before immunization with the GEX39 fusion protein; (lane 5) serum to the 44,000 M₀ ocular antigen; (lane 6) serum taken before immunization with the 44,000 M₀ ocular antigen. A 27,000 M₀ component of microfilariae is identified by the antisera to the recombinant antigens (lanes 1 and 3). Weaker immunoreactivity to a similar-sized antigen is demonstrated with the serum raised against the 44,000 M₀ ocular antigen (lane 5).

Western Blot Using Human Infection Sera

Western blot experiments were conducted with sera from 27 persons with onchocerciasis, 14 of whom had posterior segment pathology. Twelve sera derived from persons with Bancroftian filariasis and fourteen from noninfected Europeans were included in this study. Antibody concentration procedures were not used. Rather, human infection sera used for Western blot analysis were diluted 1:100. The sera were tested against the PBS, 1% Triton X-100 fraction of RPE cells, a total homogenate of retina, and an optic nerve preparation. The 44,000 M₀ antigen was clearly identified in the PBS, 1% Triton X-100 extract of RPE cells, the retinal homogenate and the optic nerve preparation by three of the human infection sera (Fig. 9). The responses of all the human infection sera were best demonstrated with the optic nerve homogenate (Fig. 10). The 44,000 M₀ antigen was not recognized by any of the 14 normal human sera tested (Fig. 10A). The antigen is seen by some persons with Bancroftian filariasis (Fig. 10B). It was identified by the majority of sera.
FIGURE 5. Micrographs demonstrating the enhancement of immunofluorescence staining of cryostat sections of bovine retina after treatment with 10% formal saline and 0.5% Triton X-100. (A) Control section treated with formal saline and Triton X-100, probed with sera taken before immunization with the EX39 fusion protein. The retinal pigment epithelium is identified by characteristic autofluorescent inclusions. (B) Untreated control section probed with serum raised to the EX39 fusion protein. There is some reactivity with the neural retina and the retinal pigment epithelium. (C) Section treated with formal saline and Triton X-100 and probed with serum raised to the EX39 fusion protein. The immunofluorescence staining of the neural retina and the retinal pigment epithelium is greatly enhanced when compared to (B). The exposure and development times were identical. Calibration bar = 50 μm.

from onchocerciasis patients without clinically described retinal or optic nerve pathology (Fig. 10C) and by all sera from persons with posterior segment pathology (Fig. 10D).

DISCUSSION

The association of *Onchocerca volvulus* infection and eye disease is based on a wealth of epidemiologic and histopathologic evidence. It is generally accepted that immune responses to dead or dying microfilariae are responsible for the development of punctate keratitis and sclerosing keratitis. The pathogenesis of posterior segment lesions has so far been obscure. Clinical studies have described chorioretinal lesions, which range from unequivocal inflammatory lesions to exclusively atrophic lesions. Rodger and Chir, in a pathologic study, described peripheral areas of choroiditis characterized by pigmented, vascular, and exudative changes. These lesions may contain microfilariae, eosinophils, lymphocytes, and plasma cells. Although acute lesions may occur, it is generally accepted that the typical picture is one of degeneration, including optic atrophy.

Microfilariae occur in the vitreous humor and choroid and have been identified in the retina in vivo and in histologic sections of most ocular tissues. However, the numbers found in the retina are apparently lower than found in other regions of the eye. Mechanical damage to the retina by microfilariae might therefore contribute less to pathology than indirect inflammatory processes or direct autoantibody reactions based on immunologic cross-reactivity of retinal and parasitic molecules. The idea that autoimmune may play a role in pathogenesis can find additional support from the observation that after drug treatment with diethylcarbamazine the degenerative ocular processes are exacerbated. This effect may be mediated by the release of microfilarial antigens and subsequent inflammatory responses. The role of immune processes in the pathogenesis of some of the complications of onchocerciasis has been established.

Recently, there has been considerable interest in the role of antibody responses directed against retinal S-antigen and IRBP in the induction of autoimmune
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FIGURE 6. Light micrographs demonstrating the localization of the cross-reactive antigen in human ocular tissues. (A) The use of sera obtained before immunization with the EX39 fusion protein did not result in any staining of retina or any other tissue. (B) Serum raised to the native 44,000 M₀ antigen and, (C, D, E, F, G, H, I, and J) serum raised to the EX39 fusion protein, resulted in specific staining of ocular tissues and cell types. (B) Intense staining of the retina was obtained with serum raised to the 44,000 M₀ ocular antigen. (C) Weaker staining, but with a similar pattern, was obtained with serum raised to the EX39 fusion protein (NFL, nerve fiber layer; OS, outer segments; RPE, retinal pigment epithelium). (D) The basal aspect of RPE cells (upper arrow), and choroidal melanocytes (lower arrow), were stained. (E) There was staining of cells surrounding the nerve bundles (arrows) and nerve axons in the optic nerve posterior to the lamina cribrosa. (F) The neural epithelium (NE) of the pars plana was intensely stained. (G) The nonpigmented epithelium (NPE) of the of the ciliary body was also intensely stained. (H) In the iris, staining was detected under the pigment epithelium (arrows). (I) There was focal staining of the sphincter muscle (arrows). (J) In the peripheral cornea there was staining of some keratocytes (solid arrows), whereas others (open arrows) were not stained. All keratocytes in the central area of the cornea were unstained. Calibration bar (50 μm) in C applies to A, B, C, and F. Calibration bar (20 μm) in D applies to D, G, and J. Calibration bar (50 μm) in E applies to E, H, and I.

FIGURE 7. Identification of a 44,000 M₀ component in various ocular tissues by Western blot. The tissue components were separated on a 10% acrylamide/sodium dodecyl sulfate gel, transferred onto nitrocellulose, and probed with serum raised to the native 44,000 M₀ antigen. (Lane 1) retina; (lane 2) choroid; (lane 3) ciliary body; (lane 4) iris; (lane 5) cornea; (lane 6) lens. A 44,000 M₀ component was detected in retina, choroid, ciliary body, but not cornea, or lens.

disease. Both S-antigen and IRBP can induce uveitis in experimental animals. It has also been suggested that antibodies with specificity for these proteins may play a role in the pathogenesis of the chorioretinopathy seen in persons infected with *O. volvulus*. Many investigators have noted an increased humoral responses to S-antigen and IRBP in the sera of onchocerciasis patients, particularly in patients with posterior pole involvement. However, no correlation between the levels of antibodies against retinal proteins and the occurrence of chorioretinitis could be found. The formation of anti-retina antibodies may be a secondary phenomenon resulting from exposure of antigens by an underlying disease process. There is no indication that the occurrence of anti-S antigen and anti-IRBP antibodies in onchocerciasis patients may be based on cross-reactivity with parasitic antigens. We suggest that immune responses to S-antigen and IRBP are unlikely to be involved in the initiation of disease. This is supported by the observation of lymphocyte responses to S-antigen and IRBP in persons with no history of uveitis.

Intravitreal injection of microfilariae of *O. lienalis* into the eyes of cynomolgus monkeys has been used as a model of posterior segment changes in onchocerciasis. Inflammatory responses to *O. lienalis* microfilariae injected intravitreally occur before any antibody reactivity and T-cell blastogenesis responses against microfilariae can be detected. The authors suggested that direct effects of microfilariae by activation of complement via the alternative pathway fulfill important roles in the pathogenesis of posterior segment lesions.

The guinea pig has been used to study acute inflammatory responses and lesions in the cornea after injection of microfilariae into the conjunctiva. These
FIGURE 8. Organ and tissue distribution of the cross-reactive antigen was determined by Western blot using murine material. The components were separated on a 10% acrylamide/sodium dodecyl sulfate gel, transferred to nitrocellulose, and probed with serum raised to the native 44,000 Mr antigen. (Lane 1) control bovine optic nerve; (lane 2) brain; (lane 3) eye—posterior segment; (lane 4) cornea; (lane 5) lens; (lane 6) skin; (lane 7) liver; (lane 8) kidney; (lane 9) spleen; (lane 10) small intestine; (lane 11) skeletal muscle; (lane 12) heart. There is strong immunoreactivity with the 44,000 Mr component of bovine optic nerve (lane 1) and a 47,000 Mr antigen of murine brain and eye (lanes 2 and 3). Weaker immunoreactivity with a 47,000 Mr component of skin and liver was detected (lanes 6 and 7).

animals develop keratitis and mount an autoantibody response to components of cornea.48 No cross-reactivity has been demonstrated between the corneal antigens and microfilariae, so an underlying disease process might trigger autoantibody formation. Autoantibody-mediated inflammatory mechanisms might contribute to the formation of chronic lesions of the cornea.

The results presented here demonstrate a specific cross-reactivity between a component of the RPE, the neural retina, the optic nerve, cells of the iris, the ciliary body, the keratocytes of the peripheral cornea, and a characterized recombinant antigen of *O. volvulus*. Not only is the ocular antigen recognized by sera raised to the EX39 fusion protein but also by sera from all persons with onchocerciasis and posterior segment disease. Western blotting using microfilarial antigens and sera to the two recombinant parasite antigens and the 44,000 Mr, ocular antigen identified a 27,000 Mr microfilarial antigen by Western blot analysis. This experiment indicates the reciprocal nature of the cross-reactivity as the microfilarial antigen was recognized, although weakly, by the serum to the 44,000 Mr, ocular antigen. The antigen identified in microfilariae by Western blotting is of greater molecular mass than that identified by immunoprecipitation of radiolabeled adult worm antigens.17 This may be attributable to the different techniques employed or differences in the expression of the antigen in adults and microfilariae.

The loss of reactivity to RPE, retina, and optic nerve after incubation of the EX39 serum with the parasite-derived recombinant antigen indicates the specificity of the cross-reactivity with the 44,000 Mr, ocular antigen. In addition, sera to the EX39 fusion protein and the semipurified native 44,000 Mr antigen yielded similar staining patterns on histologic sections. The 44,000 Mr, antigen is localized immunocytochemically to the epithelial layers of the ciliary body, the iris, and the iris sphincter muscle. These cells are of com-

FIGURE 9. Reactivity of onchocerciasis infection serum with a 44,000 Mr ocular component demonstrated by Western blot. (Lane 1) phosphate-buffered saline 1% Triton X-100 fraction of retinal pigment epithelial cells; (lane 2) total retina fraction; (lane 3) optic nerve homogenate. Samples were separated on a 10% acrylamide/sodium dodecyl sulfate gel, transferred onto nitrocellulose, and probed with human infection serum from patient 26 listed in Table 1. The serum reacts with a 44,000 Mr, component of retinal pigment epithelium, retina, and optic nerve.
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FIGURE 10. Reactivity of normal human sera and infection sera with a 44,000 M_r ocular component demonstrated by Western blot. The onchocerciasis infection sera used were taken from patients whose clinical findings are summarized in Table 1. Components of bovine optic nerve homogenate were separated on 10% acrylamide/sodium dodecyl sulfate gels under reducing conditions, transferred onto nitrocellulose, and probed with: (Panel A) 14 European control sera; (Panel B) 12 Bancroftian filariasis infection sera; (Panel C) 11 onchocerciasis infection sera from persons without posterior segment disease; (Panel D) 16 onchocerciasis infection sera from persons with posterior segment disease. The extreme right lane in each panel has been probed with rabbit serum raised to the EX39 fusion protein. Normal human sera show no reactivity with the 44,000 M_r component (panel A). Sera from some persons with Bancroftian filariasis (panel B), and onchocerciasis without posterior segment disease (panel C), recognize a 44,000 M_r component of optic nerve. Immunoreactivity to a 44,000 M_r component is present in sera of all persons with onchocerciasis and posterior segment disease (Panel D).

mon embryologic origin. There was some staining of stromal keratocytes in the anterior aspect of the cornea at the limbus. This may correlate with the development of sclerosing keratitis. However, this finding has not been supported by Western blot analysis. The 44,000 M_r cross-reacting ocular antigen was detected in optic nerve, RPE, retina, choroid, ciliary body, and iris by Western blot analysis. The tissue distribution of the cross-reacting antigen in other organs has been determined using murine material. An antigen of 47,000 M_r was detected in brain and eye and may be present in skin and liver. This antigen was also recognized by sera to the EX39 and GEX39 fusion proteins, confirming the presence of the cross-reacting epitope.

The tissue distribution of the 44,000 M_r antigen correlates with the sites of ocular pathology. Involve-
ment of the 44,000 M, antigen in the pathogenesis of ocular onchocerciasis may explain observed physiologic peculiarities. Some onchocerciasis patients have unusually low intraocular pressure\(^{30,51}\) and pigment dispersion from the iris. It has been suggested that atrophy of the ciliary body may result in reduced production of aqueous and consequently a lower intraocular pressure.\(^{51}\) The ciliary epithelium may be a target of immune attack due to the presence of the cross-reacting antigen. Immunologic cross-reactivity may also explain the development of pathology in the iris. Both the loss of pigment\(^{30,55}\) and the miosis, sometimes pear-shaped, sluggish pupil characteristic of onchocerciasis\(^{59}\) may be explained by the destruction of the iris epithelium, the pigment cells of the iris stroma, and the iris musculature. Chronic uveitis may ultimately lead to extensive anterior synechiae and secondary glaucoma.\(^{54}\) The existence of the cross-reacting antigen in the retina, RPE, choroidal melanocytes, and optic nerve may account for the unusual chorioretinal degeneration and optic neuritis/atrophy.

Marshall and Cherry\(^{35}\) suggested that microfilariae are involved in the development of Nakalanga dwarfism by affecting the pituitary gland. In a more recent study, it was speculated that inflammation in the central nervous system may contribute to seizures in onchocerciasis patients living in an endemic area of Uganda. This suggestion was based on the observation of improvement in seizures (grand and petit mal) after ivermectin treatment.\(^{56}\) The identification of the cross-reacting antigen in neural tissues may be of importance in light of these reports.

Southern blot experiments identified DNA sequences with homology to Ov39 in other filarial nematodes which have not been associated with ocular disease. DNA from *Wuchereria bancrofti*, another human filarial parasite, is not easily obtained. However, in Western blot experiments, sera from persons with Bancroftian filariasis contain antibodies reactive with the 44,000 M, antigen suggesting the presence of a shared epitope. It should be noted that the cross-reactive antibodies were not concentrated by affinity purification, rather these sera were used in a 1:100 dilution. These results indicate a very significant titer of circulating antibodies to the cross-reacting epitope in the sera of persons with filarial infections.

The question of why one filarial nematode should cause ocular disease while another does not may be related to the behavior of the microfilariae and the location of the cross-reacting ocular antigen. We suggest that onchocerciasis is unusual. In contrast to other filarial diseases, in onchocerciasis, microfilariae occur in vivo in the corneal stroma,\(^{37}\) the anterior chamber,\(^{38}\) the vitreous humor,\(^{39}\) and the retina.\(^{39}\) In histologic sections they have been identified in the cornea, anterior chamber, iris, ciliary body, vitreous humor, choroid, retina, and optic nerve.\(^{51}\) With regard to the cross-reacting ocular antigen, the greatly enhanced immunofluorescence staining of cryostat sections after treatment with formalin and Triton X-100 suggests the cross-reactive epitope may be masked by lipid. Inflammatory responses to dying microfilariae in the eye will damage adjacent ocular tissues possibly exposing the cross-reactive epitope to the immune system. Exacerbation of disease may be promoted by complement activation or cytotoxic cells of the immune system. Alternatively, cells of the eye may present the cross-reacting epitope in the context of MHC class II as a consequence of inflammation. MHC class II expression by RPE cells induced by lymphokines has been demonstrated in vitro.\(^{60,61}\) An increase in the in vivo expression of MHC class II antigens has been documented for some cells of the iris in onchocerciasis.\(^{7}\) The presented cross-reactive epitope in the presence of existing strong anti-parasite responses could be an unintentional target of immune attack.

The induction of uveitis in experimental models has been demonstrated using exogenous peptides derived from various viral and bacterial proteins with similar sequences to peptide M, the uveitopathogenic peptide of S-antigen.\(^{62-64}\) However, epidemiologic evidence for the causal association of uveitis and infection with viral or bacterial infection is lacking. Ocular onchocerciasis may be the first example of a human filarial disease where immunologic cross-reactivity can be implicated in the pathogenesis. The extensive epidemiologic evidence associating *O. volvulus* infection with ocular disease and the demonstration of strong serologic responses to the cross-reacting ocular antigen and the appropriate tissue distribution of the cross-reacting ocular antigen support this concept.

**Key Words**

onchocerciasis, filariasis, cross-reactivity, autoimmunity, pathogenesis

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**References**


