Autoantibody Induced by Experimental Onchocerca Infection

Effect of Different Routes of Administration of Microfilariae and of Treatment With Diethylcarbamazine Citrate and Ivermectin

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Hartley guinea pigs were injected with microfilariae (Mf) of Onchocerca lienalis as a model for acute inflammatory responses to Mf in human Onchocerca volvulus infection. IgG autoantibody reactive with a 3 M KCl extract of guinea pig cornea was detected by ELISA in the serum of guinea pigs injected with O. lienalis Mf three or more times subconjunctivally, or two or more times subcutaneously. Administration of the microfilaricides diethylcarbamazine citrate and ivermectin did not alter the proportion of animals expressing autoantibody or the mean autoantibody titer. The severity of acute corneal inflammatory reactions to Mf was similar in animals with and without circulating autoantibody. Although autoantibody responses did not correlate with acute corneal inflammatory reactions to dead Mf, the ability of Mf to induce formation of an antibody reactive with a component of autologous cornea suggests that autoimmune mechanisms might participate in chronic ocular lesions in the cornea, eg, sclerosing keratitis. Invest Ophthalmol Vis Sci 29:827-831, 1988

Onchocerca volvulus infection afflicts an estimated 30 to 50 million humans worldwide. In hyperendemic areas, 10% of all persons infected and 50% of adults over 40 may be blind due to corneal or retinal inflammatory lesions caused by O. volvulus.1 Corneal disease in onchocerciasis may occur as punctate keratitis or sclerosing keratitis. Punctate keratitis consists of greyish-white opacities composed of acute inflammatory cells and localized edema which form around dying or dead microfilariae (MF).1 These opacities resolve without leaving obvious permanent damage when the microfilarial debris has been degraded by the inflammatory cells. Sclerosing keratitis consists of a fibrovascular pannus which usually begins from the inferior and temporal limbus, and progresses to cover the entire cornea.1 Punctate keratitis is more often found in light infections of shorter (less than 10 years) duration, while sclerosing keratitis is associated with heavy infections of longer (10 years or more) duration.1 Immunopathologic mechanisms mediated by IgE antibody and eosinophils are thought to be of importance in punctate keratitis, while the mechanisms responsible for sclerosing keratitis are largely unknown.2-5

In order to identify the immunopathologic mechanisms responsible for the corneal inflammatory lesions of onchocerciasis, we have developed an animal model using Mf of Onchocerca lienalis, a parasite of cattle closely related to O. volvulus.2-4 Subconjunctival injection of O. lienalis Mf in guinea pigs resulted in penetration of Mf to the central cornea, and formation of opacities resembling the punctate keratitis of human onchocerciasis around individual Mf when these Mf die.4,5 Enumeration of the punctate corneal lesions and measurement of corneal neovascularization associated with the inflammatory reactions has been used to quantitate the effects of microfilaricidal drugs and of anti-inflammatory agents on the acute corneal reactions to dead Mf.5

It has been suggested that autoimmune mechanisms may be involved in the development of ocular lesions in onchocerciasis.1 Experimental studies in rabbits have demonstrated that autoimmunization with a 3 M KCl extract of autologous cornea, followed by intracorneal injection of lymphokines, can induce severe corneal inflammation.6 In the present study serum IgG antibody responses to a 3 M KCl extract of guinea pig cornea were examined in guinea pigs injected with O. lienalis Mf. Autoantibody was detected by ELISA in sera from guinea pigs given subconjunctival injections of Mf at 3-4-week intervals over a period of 14 weeks, or subcutaneous injections of Mf at two week intervals over 4 weeks. No obvious correlations were found between autoantibody levels and acute corneal inflammatory reactions to Mf, or between serum autoantibody responses and treatment with microfilaricidal drugs. The ability of Mf in extraocular sites to induce autoantibody reactive with cornea suggests that autoimmune chronic corneal inflammatory disease could occur in human onchocerciasis, for example in long-standing infections.

Materials and Methods. Microfilariae (Mf) of Onchocerca lienalis were obtained from the umbilical skin of cattle, cryopreserved, and prepared for injection as previously described.2,4 Groups of 20-30 female Hartley guinea pigs (500-600 g body weight)
were given subconjunctival injections of 0.1 ml of Dulbecco’s Modified Eagle’s medium (DME) containing 5000 Mf in the superior bulbar conjunctiva of both eyes, or two subcutaneous injections of 5000 Mf followed by one intracorneal injection of 5000 Mf in the right eye. Control animals received bilateral subconjunctival injections of 0.1 ml of DME, or subcutaneous injections of 0.5 ml of DME. All experiments were carried out in accordance with the ARVO Resolution on the Use of Animals in Research.

Drug treatments were given following the subconjunctival injections of Mf. Betamethasone (Schering Pharmaceuticals, Kenilworth, NJ) was given to treatment groups of six to ten animals at a dose rate of 0.1 mg/kg daily beginning 4 days after the subconjunctival injection of Mf. Diethylcarbamazine citrate (DEC, Sigma Chemical Co., St. Louis, MO) was given at 15 mg/kg daily beginning 5 days after the injection of Mf. Betamethasone and DEC were diluted in sterile 0.01 M phosphate-buffered 0.15 N NaCl, pH 7.4, (PBS) and administered by subcutaneous injection. A single dose of 0.2 mg/kg of ivermectin diluted in PBS, was given orally 5 days after injection of Mf. Drug treatments and clinical examinations were conducted in a double-masked fashion. Clinical examinations were performed with a Zeiss slit lamp before the injection of Mf, and 2, 4, 7 and 8 days after injection, as previously described.

Antigen extracts were prepared from O. lienalis Mf and normal guinea pig cornea. O. lienalis Mf were disrupted by freeze-thawing and sonication in PBS, centrifuged for 5 min at 8000 g, and supernatants were stored in liquid nitrogen. Guinea pig cornes, with epithelium attached, were stored in 3 M KCl for 2 days at 4°C, centrifuged for 10 min at 8000 g, and dialyzed against PBS. The protein content of the Mf and guinea pig cornea extracts was determined by the Coomassie blue dye-binding assay of Bradford standardized with bovine serum albumin.

Enzyme-linked immunosorbent assays (ELISA) were performed using Immulon 2 round-bottomed 96-well polystyrene plates (Dynatech, Alexandria, VA) coated with O. lienalis Mf antigen (1 µg/well), or guinea pig cornea antigen (5 µg/well), in 0.1 M sodium bicarbonate buffer, pH 9.0, for 18 hr at 4°C. Sera were added at 1:700 dilution in PBS plus 10% FBS for anti-O. lienalis antibody assays, or undiluted for autoantibody assays, and incubated for 2 hr at room temperature, or at 37°C. Peroxidase-conjugated goat anti-guinea pig IgG (Cooper Biomedical, King of Prussia, PA) was added at 1:200 dilution and incubated for 1 hr at room temperature, or at 37°C. Substrate development was carried out for 30 min at room temperature using 0.5 mg/ml 5-aminosalicylic acid, pH 6.1. The optical density at 492 nm (OD492) was determined using a Multiskan MC ELISA reader (Flow Laboratories, McLean, VA) with an Apple Ile (Cupertino, CA) computer interface.

Statistical analyses were carried out by one-way analysis of variance, by the chi-squared test, and by the Pearson product-moment correlation.

Results. ELISA for IgG antibody were performed on serum using O. lienalis Mf extract or 3 M KCl extract of guinea pig cornea as antigen. Thirty-two normal sera from guinea pigs aged 2 to 5 months gave ELISA optical densities at 492 nm of 0.043 ± 0.040 (SD) against the cornea extract when incubations were conducted at room temperature. When ELISA incubations were conducted at 37°C, the average OD492 for normal sera was 0.297 ± 0.128. Sera were considered positive for autoantibody if the OD492 against corneal extract was equal to or greater than the mean plus 3 SD for normal serum determined under identical conditions.

The time course of expression of IgG autoantibody and antibody to O. lienalis in 22 animals injected subconjunctivally with O. lienalis Mf is shown in Figure 1A. Injections of Mf were given on days 0, 27, 68 and 84, and DEC, DEC plus betamethasone, or no treatment was given on days 31-35, 71-75, 85-90. The combined data for all treatment groups are shown in Figure 1A. Autoantibody was not detected until 12 days after the third subconjunctival injection of Mf, while anti-O. lienalis antibody was present after one injection of Mf. Figure 1B shows the time course of expression of anti-O. lienalis IgG antibody and autoantibody following two subcutaneous injections and one intracorneal injection of Mf in 20 guinea pigs. Subcutaneous injections were given on days 0 and 14 and the intracorneal injection was given on day 28. Autoantibody appeared in 10 of 20 animals after one subcutaneous injection and in 18 of 20 after two subcutaneous injections (Fig. 1B). No intracorneal Mf were seen in these animals prior to administration of the intracorneal challenge injection. The peak IgG antibody response against O. lienalis was approximately half that observed in animals given subconjunctival injections of Mf. Animals injected subconjunctivally or subcutaneously with DME did not develop detectable autoantibody.

Guinea pigs given subconjunctival injections of Mf had microfilariae in the central cornea within 2 days, and subsequently developed inflammatory reactions as these Mf lost motility and appeared to die. The number of punctate opacities containing nonmotile, degenerating Mf, and the extent of corneal neovascularization, were estimated. The relationship between the numbers of punctate opacities and the presence or absence of autoantibody in the experiment shown in Figure 1A is shown in Table 1. No significant dif-
ference in the number of opacities was observed between animals with or without autoantibody. The Pearson product-moment correlation was used to determine whether any association existed between the amount of autoantibody (ELISA OD492) and the number of punctate opacities, the length of corneal new vessels, or the amount of anti-O. lienalis antibody (ELISA OD492). A weak (r = 0.39) but statistically significant (P < 0.05) positive correlation was found between the autoantibody and anti-O. lienalis antibody titers. No other correlations could be demonstrated.

The effects of treatments with microfilaricides and anti-inflammatory drugs on the autoantibody response were studied in guinea pigs injected subconjunctivally with O. lienalis Mf. Figure 2 shows the autoantibody responses of three groups of guinea pigs given subconjunctival injections of Mf on days 0, 27, 68 and 84, and treated with DEC, DEC plus betamethasone, or not treated. The combined data for all treatment groups are shown in Figure 1A. Figure 3 shows the autoantibody responses of three groups of guinea pigs given subconjunctival injections of Mf on days 0, 27, 68 and 84, and treated with DEC on days 37–39 and 92–96, with ivermectin on days 37 and 92, or not treated. Using the chi-squared test, no statistically significant differences in the proportion of autoantibody-positive animals were found between the groups in either experiment. In both experiments, the effect of drug treatment on the magnitude of the autoantibody response (estimated by using the ELISA OD492) was estimated by one-way analysis of variance. No statis-

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**Table 1.** Mean punctate opacities/animal ± SEM for guinea pigs with and without autoantibody on day 89

<table>
<thead>
<tr>
<th>Day</th>
<th>With auto Ab (n = 5)</th>
<th>Without auto Ab (n = 9)</th>
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<tbody>
<tr>
<td>74</td>
<td>31 ± 4.5</td>
<td>31 ± 3.9</td>
</tr>
<tr>
<td>76</td>
<td>17 ± 3.3</td>
<td>25 ± 4.2</td>
</tr>
<tr>
<td>86</td>
<td>27.9 ± 6.3</td>
<td>24 ± 3.6</td>
</tr>
<tr>
<td>88</td>
<td>27 ± 3.0</td>
<td>27 ± 2.5</td>
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Mf injections were given on days 0, 27, 68, 84; DEC, DEC plus betamethasone, or no treatment were given on days 31–35, 71–75 and 85–90.

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**Fig. 1.** (A) Anti-O. lienalis and anti-guinea pig cornea autoantibody following subconjunctival injection of Mf. ○, ELISA OD492 (×1000) for undiluted serum tested against 3 M KCl extract of guinea pig cornea, for all treatment groups combined. ●, mean of the ELISA OD492 (×1000) for sera from all groups, diluted 1:50 and tested against O. lienalis Mf extract. Incubations were carried out at room temperature. Error bars denote ±SEM. Dashed line denotes minimum positive OD492 value for autoantibody at 25°C. Subconjunctival injections of 5000 Mf were given on days 0, 27, 68 and 84; DEC, DEC plus betamethasone, or no treatment, were given on days 31–35, 71–75, 85–90. (B) Anti-O. lienalis and anti-guinea pig cornea autoantibody following subcutaneous and intracorneal injection of O. lienalis Mf. ○, ELISA OD492 (×1000) for undiluted serum tested against 3 M KCl extract of guinea pig cornea, ●, mean ELISA OD492 (×1000) for serum diluted 1:50 and tested against O. lienalis Mf extract. Incubations were carried out at room temperature. Error bars denote ±SEM. Dashed line denotes minimum positive OD492 value for autoantibody at 25°C. Subcutaneous injections of 5000 Mf were given on days 0 and 14, and an intracorneal injection of 5,000 Mf was given on day 28 (arrows).
autoantigens cross-react with an excretory-secretory product of Mf not present in somatic Mf extracts. It also is possible that an autoantigen cross-reacting against simple cross-reactivity between host cornea and Mf antigens. The origin of the immunogens which induced the autoantibodies against corneal antigens is unknown.

Discussion. In the present study we have examined the potential role of serum IgG antibody reactive with a 3 M KCl extract of guinea pig cornea, induced by infection with Mf of Onchocerca lienalis, in acute corneal inflammatory responses to Mf. No apparent correlations were found between the presence of autoantibody in the serum and the magnitude of localized acute inflammatory reactions around intracorneal Mf, estimated by counting of punctate opacities and by measurement of corneal neovascularization. The magnitude of the autoantibody response correlated with the titer of anti-O. lienalis IgG antibody, and the proportion of animals expressing autoantibody increased with successive injections of Mf. Neither DEC nor ivermectin treatment increased the autoantibody response, and betamethasone given after injection of Mf did not affect the autoantibody response.

The origin of the immunogens which induced the autoantibodies against corneal antigens is unknown. The absence of detectable anti-cornea autoantibody from some sera containing high levels of anti-O. lienalis IgG antibody (eg, day 70 in Fig. 1A) argues against simple cross-reactivity between host cornea and somatic Mf antigens. It may be that the corneal autoantigens cross-react with an excretory-secretory product of Mf not present in somatic Mf extracts. It also is possible that an autoantigen cross-reacting with cornea is released from collagenous tissues during migration of Mf through skin or conjunctiva, possibly by proteolytic enzymes of microfilarial origin.

Human onchocercal keratitis may appear as reversible local acute inflammatory foci around individual Mf (punctate keratitis), or as a permanent fibrovascular pannus which progresses centripetally from the limbus (sclerosing keratitis). Sclerosing keratitis is the leading cause of blindness due to onchocerciasis in savanna regions of West Africa. Punctate keratitis and sclerosing keratitis are not generally seen in the same individuals, with sclerosing keratitis being associated with more advanced disease of longer duration. The present studies suggest that autoantibody to corneal antigens may not contribute to the acute punctate keratitis-like lesions of the guinea pig model. The importance of autoimmune mechanisms in the chronic inflammatory processes of sclerosing keratitis remains to be determined. The ability of Mf in extracocular sites to induce autoantibody reactive with a component of cornea suggests that significant autoimmune corneal disease could occur in human onchocerciasis.

Key words: onchocerciasis, experimental model, cornea, autoantibody, punctate keratitis

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References


