Humoral Autoimmune Response Against S-Antigen and IRBP in Ocular Onchocerciasis

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Autoimmune mechanisms are thought to play a role in the pathogenesis of the chorioretinal changes in ocular onchocerciasis. In this study the involvement of autoimmunity against retinal antigens in developing chorioretinitis was investigated. Serum levels of autoantibodies, directed against human S-antigen and interphotoreceptor retinoid-binding protein (IRBP), were determined in patients with onchocerciasis (n = 46) and endemic controls (n = 38) from Sierra Leone with the use of an enzyme immunoassay. In both groups high levels of anti-human S-antigen and IRBP antibodies were detected. No relationship could be demonstrated between the antiretinal antibody level and the occurrence of chorioretinitis in onchocerciasis. The levels of both anti-human S-antigen and IRBP antibodies were significantly higher in patients with onchocerciasis compared with endemic controls (P < 0.001). Cross-reactivity of antiretinal antibodies with parasitic antigens could not be demonstrated as a possible explanation for the higher levels in patients with onchocerciasis. No correlation was found between the levels of antibodies of different classes against the crude Onchocerca volvulus, the egg antigen, or the microfilariae and the antiretinal antibody levels. Furthermore, in a panel of 13 different monoclonal antibodies directed against O. volvulus, only one showed a slight anti-human IRBP reactivity and none reacted with S-antigen. The immune response against the two retinal antigens investigated was not specific for onchocerciasis because high antibody levels were also found in patients with Bancroftian filariasis from Papua, New Guinea, and Surinam. The finding of retinal autoimmunity in a selected group of people from West Africa could well be explained by the induction of polyclonal B cell activation resulting from parasitic infection. Circulating antibodies against human S-antigen or human IRBP are thus not specific for onchocerciasis and are in themselves not sufficient to cause chorioretinopathy in onchocerciasis, although their pathogenic role in an ongoing chorioretinitis cannot be excluded.

Ocular onchocerciasis is a major cause of blindness in Africa and some parts of Central and South America. Up to 85 million people are exposed to the risk of infection with the nematode Onchocerca volvulus, which is transmitted by flies of the Simulium species. Worldwide, an estimated one million people have significant visual loss, leading to at least partial disability as a result of onchocerciasis. In most cases this visual loss results from corneal scarring or pathologic changes in the retina, choroid, or optic nerve or in a combination of these.1,2 Ocular onchocerciasis clinically presents as punctate keratitis, sclerosing keratitis, irisocyclitis (with or without secondary glaucoma), degenerative chorioretinitis, and optic atrophy.

The punctate keratitis develops as an inflammatory reaction to dead microfilariae. Histologic examination of these lesions shows necrotic parasites in the corneal stroma, surrounded by lymphocytes and eosinophils.3 However, little is known about the natural history of the chorioretinitis.1,4,5 Although living microfilariae have been detected in the retina in vivo and demonstrated histologically in retinal tissue,7 the evidence of onchocerciasis as an etiologic factor remains largely epidemiologic.

One explanation may be that initially the disease process starts with a choroiditis and is followed by a secondary degeneration of the overlying pigment epithelium and neuroretina, in which the atrophic lesion is possibly an end stage. This hypothesis is supported by experimental studies in the rabbit.3 In this experimental model the extent of the atrophy is often disproportionate to the histologic evidence of uveitis. This suggests that other factors, besides an inflammatory reaction to in situ microfilariae, are involved. Numerous hypotheses have been put forward con-
cerning the pathogenesis of the chorioretinopathy. Autoimmunity is one of the factors that are thought to play a role in the subsequent events leading to atrophy of the pigment epithelium and choriocapillaris. New data appear to confirm the hypothesis of an autoimmune pathogenesis. Antibodies against bovine retinal S-antigen have been detected in patient sera with the aid of an enzyme-linked immunosorbent assay technique (ELISA) and a human basophil degranulation test (HBDT).8 We have extended these experiments by investigating the antibody response against two well-defined retinal proteins, S-antigen and interphotoreceptor retinoid-binding protein (IRBP). The uveitogenicity of these proteins has been demonstrated in a variety of animal species, including primates.9 In the study presented here we used antigens isolated from human retinas in view of the difference in antigenicity between human and bovine retinal proteins.10,11

Materials and Methods

Patients and Controls

In 1985 and 1986 blood was obtained from patients with onchocerciasis (n = 46) and from endemic controls (n = 38) in Sierra Leone, West Africa. These two groups were subdivided on the basis of the skin-snip test results and the reaction within 24 hr after ingestion of a test dose of 50 mg diethylcarbamazine (Mazzotti test). People with positive skin-snip test results or positive Mazzotti test results were considered to have onchocerciasis. People with negative skin-snip-test results and negative Mazzotti test results were accepted as endemic controls.

None of the patients was being treated with diethylcarbamazine (DEC), suramin, or corticosteroids at the time a blood sample was drawn, although some of them had been treated with these drugs in the past. The two groups were examined according to the Onchocerciasis Survey Form.12 A summary of their ocular status is shown in Table 1. A number of symptoms were not visible, because of opacities in the anterior segment. Chorioretinitis was scored in case of early disruption of retinal pigment alone or with exposure of the choroid and clumping of retinal pigment at any degree. Venous blood (10 ml) was drawn from each person. Frozen serum was transported to the Netherlands in iceboxes. There were three other control groups in addition to the endemic controls from Sierra Leone. Two groups consisted of patients with a Bancroftian filariasis from Papua, New Guinea (n = 22), and Surinam (South America) (n = 28). Their serum was collected in 1987 and kept frozen at −20°C until use. A third control group consisted of healthy Dutch people (n = 25). Informed consent of patients and controls was obtained after the study was explained.

Table 1. Ophthalmologic features in onchocerciasis patients and endemic controls from Sierra Leone

<table>
<thead>
<tr>
<th>Ocular status</th>
<th>Onchocerciasis patients* (n = 46)</th>
<th>Endemic controls† (n = 38)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OD</td>
<td>OS</td>
</tr>
<tr>
<td>Visual acuity (&lt;6/60)</td>
<td>13/46</td>
<td>15/46</td>
</tr>
<tr>
<td>IOP (&gt;22 mmHg)</td>
<td>8/46</td>
<td>9/46</td>
</tr>
<tr>
<td>MF/cornea</td>
<td>6/45</td>
<td>9/46</td>
</tr>
<tr>
<td>MF/AC</td>
<td>14/43</td>
<td>18/46</td>
</tr>
<tr>
<td>Snowflake keratitis</td>
<td>20/43</td>
<td>20/46</td>
</tr>
<tr>
<td>Sclerosing keratitis</td>
<td>6/43</td>
<td>8/46</td>
</tr>
<tr>
<td>Iritis</td>
<td>8/41</td>
<td>11/45</td>
</tr>
<tr>
<td>Lens cataract:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early</td>
<td>6/39</td>
<td>7/42</td>
</tr>
<tr>
<td>Advanced</td>
<td>1/39</td>
<td>2/42</td>
</tr>
<tr>
<td>Mature</td>
<td>1/39</td>
<td>1/42</td>
</tr>
<tr>
<td>Posterior segment changes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Retina/choroid</td>
<td>6/36</td>
<td>10/40</td>
</tr>
<tr>
<td>Optic nerve (except cupping)</td>
<td>7/39</td>
<td>6/39</td>
</tr>
<tr>
<td>Optic nerve/cupping</td>
<td>5/39</td>
<td>5/39</td>
</tr>
</tbody>
</table>

* Age (yr): mean, 36.6; range 18–70.† Age (yr): mean, 30.5; range 11–60. Data represent number of affected eyes over total eyes evaluated. In a number of cases symptoms were not visible, because of opacities in the anterior segment.

Monoclonal Antibodies Against O. volvulus

The procedure for isolating the cuticle and the internal organs such as intestine and uterus from the adult worms was as follows. Adult worms, derived from a nodule of an patient with onchocerciasis from Sierra Leone, underwent a microdissection to separate the cuticle and the internal organs.13 A modification was introduced for separating the eggs from the microfilariae by means of filtration over plankton-gauze with a pore size of 15 μm.

Monoclonal antibodies (mAbs) were produced, with the use of the method developed by Köhler and Milstein.14 Eight mAb preparations were directed against the surface of the egg of the O. volvulus and another five mAbs against the epicuticle of the adult parasite. In this article the mAbs used were mouse ascites fluid, in a final dilution of 1/20.

A mouse IgG1, monoclonal PDS1, directed against porcine S-antigen (kindly provided by Dr. J. P. Banga, London, England), was used as a positive control on the reagents in the anti-human S-antigen ELISA (see below). In the anti-human IRBP ELISA, we took a rabbit anti-human IRBP antiserum diluted 1/1000 in phosphate-buffered saline (PBS)-Tween 20® (OPG Farma, Utrecht, The Netherlands) as a positive control.
Human S-Antigen and Human IRBP

Human S-antigen and human IRBP were isolated from retinas of human cadaver eyes from which the corneas had been removed for transplantation. These retinas were stored at −20°C until use.

The isolation of human S-antigen was performed as described by Doekes et al. A 50% ammonium sulfate precipitation of retinal extract was followed by DEAE (DE-52, Whatman Corporation, Kent, United Kingdom) anion exchange chromatography and gel filtration.

Human IRBP was purified with the use of a modification of the method described by Adler et al. After thawing, the retinas were extracted with cold PBS containing 0.1 mM phenylmethyl-sulfonyl fluoride (PMSF; Sigma, St. Louis, MO) and 0.02% NaN₃, pH 7.4, for 1 hr at 4°C. After centrifugation for 30 min at 3,000 g, the pellet was resuspended in the same solution and the extraction procedure was repeated. Both supernatants were collected and concentrated by ultrafiltration on an XM-50 Diaflo® ultrafiltration membrane (Amicon Corporation, Danvers, MA). Further isolation was performed by 30% ammonium sulfate precipitation and affinity chromatography on a Concanavalin A-Sepharose® column (Pharmacia AB, Uppsala, Sweden) in PBS, containing 0.5 M NaCl, 0.2 mM PMSF, and 0.02% NaN₃, pH 7.4, for 1 hr at room temperature. After they were washed three times with PBS containing 0.1% (v/v) Tween 20® (PBS-Tween®) the wells were incubated with 0.1 ml serum diluted 1/20 in PBS-Tween®. All sera were tested in coated and uncoated wells as a control for a specific binding. After 1 hr at room temperature, the plates were washed and 0.1 ml rabbit anti-human IgG, IgM, IgA, kappa, lambda antiserum conjugated with horseradish peroxidase (DAKOPATTS, Copenhagen, Denmark) diluted 1/500 in PBS-Tween® was added. After incubation for 1 hr at room temperature and three washes with PBS-Tween®, the plates were incubated for 30 min at room temperature with 0.1 ml 0.20 mM ABTS (Boehringer, Mannheim, Federal Republic of Germany) in 0.05 M citric acid (pH 4.0) containing 0.15% H₂O₂. The green reaction product was measured in a spectrophotometer at 405 nm.

To detect antibodies of the IgE class against human S-antigen and IRBP, a rabbit anti-human IgE antiserum conjugated with horseradish peroxidase (Nor- dic, Tilburg, The Netherlands) was used instead of rabbit anti-human IgG in the same dilution.

In each plate at least five coated and five uncoated wells were incubated with PBS-Tween® instead of serum, to correct for the amount of rabbit anti-human IgG-PO or rabbit anti-human IgE-PO directed against human S-antigen or human IRBP.

Calculations were made as follows:

\[
(A_{a,t} - A_{c,t}) - (A_{a,o} - A_{c,o})
\]

in which
\[
A_{a,t} = \text{mean Extinction at 405 nm in S-antigen or IRBP coated wells incubated with test serum;}
\]
\[
A_{c,t} = \text{mean E 405 nm in uncoated wells incubated with the same test serum;}
\]
\[
A_{a,o} = \text{mean E 405 nm in S-antigen or IRBP coated wells incubated with PBS-Tween®;}
\]
\[
A_{c,o} = \text{mean E 405 nm in uncoated wells incubated with PBS-Tween®.}
\]

The expression \((A_{a,o} - A_{c,o})\) in the formula was 0.009 ± 0.006 for human S-antigen and 0.105 ± 0.019 for human IRBP.

In the anti-human S-antigen ELISA a reference serum was always included as a positive control. This serum, from a healthy Dutch person, contained a relatively high level of antibodies directed against human S-antigen.

The possibility of an amplification of the ELISA system by rheumatoid factor in the tested sera was also investigated. A serum, which contained a high
level of rheumatoid factor of the IgM class, was added to the reference serum in the anti-human S-antigen ELISA in a dilution with a moderate extinction. No elevation of the original extinction was observed, which meant that interference of rheumatoid factor in our assay system was not likely. The reference serum itself did not contain rheumatoid factor.

In the anti-human IRBP ELISA, only a rabbit anti-human IRBP antiserum was used as a positive control because no patients or healthy Dutch people were found to have significant amounts of anti-human IRBP antibodies.

A serum was considered negative if the corrected extinction was lower than 0.100.

The statistical analysis of data was performed with the use of the Mann-Whitney rank-sum test.

**Results**

The role of autoimmunity against retinal proteins in onchocerciasis was investigated by measuring antibodies against human S-antigen and human IRBP in serum. High levels of anti-human S-antigen antibodies were detected in all sera of patients with onchocerciasis and in endemic controls from Sierra Leone (Fig. 1). In both groups a considerable range of values was recorded. The level of anti-human S-antigen antibodies in patients with onchocerciasis (mean: 0.462) was significantly higher than in the endemic controls (mean: 0.336; Mann-Whitney rank-sum test; \( P < 0.001 \)). In the control group made up of healthy Dutch people, in 32% an extinction of more than 0.100 was recorded, but the mean value (0.084) of anti-human S-antigen antibody activity was much lower than in the groups from Sierra Leone.

To ascertain whether the level of anti-S-antigen antibodies was related to posterior segment involvement, we divided the onchocerciasis group into patients with and without chorioretinitis. Of the 46 patients, 10 had clear-cut evidence of chorioretinal changes in one or both eyes (Table 1). The fundus in ten right eyes and six left eyes could not be examined because of anterior segment opacities, whereby both eyes were involved in five patients. Eyes with opaque media were considered to be chorioretinitis negative. There was no difference between the anti-human S-antigen levels in patients with and those without chorioretinitis (Fig. 2), nor could a correlation be demonstrated between the anti-human S-antigen antibodies and the number of microfilariae in the cornea, anterior chamber, or skin-snip (data not shown).

To determine whether the presence of high serum levels of anti-S-antigen in onchocerciasis resulted from cross-reaction of retinal S-antigen with parasitic antigens, the following experiments were performed. All sera of patients with onchocerciasis were tested for separate IgG, IgM, IgA, and IgE antibodies against the crude *O. volvulus*, the egg antigen, and microfilariae. No correlation could be demonstrated between the levels of the antibodies mentioned above and the concentration of antibodies against human S-antigen (data not shown).

Cross-reactivity between the parasite and human S-antigen was also studied in the following manner. Thirteen different mAbs directed against the egg surface or the epicuticle of the adult *O. volvulus* were tested for anti-human S-antigen activity. None of these mAbs reacted with human S-antigen (Fig. 3).

As we have seen, the levels of anti-human S-antigen were higher in patients with onchocerciasis than in the endemic controls (Fig. 1). To investigate whether this may be seen as a phenomenon specific to onchocerciasis or as a general finding in filarial diseases, we tested two groups of patients with Bancroftian filariasis, a disease without the ocular symp-
Onchocerca volvulus with human S-antigen and IRBP. A mouse anti-porcine S-antigen monoclonal antibody (PDS1) and a rabbit anti-human IRBP antiserum (RahulRBP) were taken as positive controls.

Symptoms seen in onchocerciasis. The sera of patients from Papua, New Guinea, with Bancroftian filariasis also showed high levels of antibodies directed against human S-antigen; all sera had detectable levels and the mean extinction was 0.359. Patients from Surinam with Bancroftian filariasis, however, had a much lower level of anti-human S-antigen antibodies; only 21% of the sera had an extinction of more than 0.100 and the mean was 0.091.

When tested for human IRBP autoantibodies, the five groups (patients with onchocerciasis, endemic controls, two groups of patients with Bancroftian filariasis, and healthy Dutch controls) showed a pattern similar to that of human S-antigen (Fig. 4). There were a few exceptions. In the onchocerciasis group 2% and in the endemic control group 8% had subdetectable levels of antibodies against human IRBP. Furthermore, the anti-human IRBP antibody levels in the group of patients from Papua, New Guinea, with Bancroftian filariasis (mean: 0.555) were significantly higher than those of the patients with onchocerciasis (Mann-Whitney rank-sum test; \( P < 0.05 \)). As with S-antigen, no relation could be demonstrated between the levels of anti-human IRBP antibodies and the occurrence of chorioretinitis (Fig. 2). No relationship could be demonstrated between the anti-IRBP antibodies on one side and antibodies directed against the crude O. volvulus, the egg-antigen or the microfilariae (whether it concerned IgG, IgM, IgA, IgE antibodies) on the other side. One of the 13 mAbs raised against parasitic antigens showed a slight anti-human IRBP activity (Fig. 3). This was an mAb directed to the surface of the egg of the parasite.

In people from Sierra Leone (both patients with onchocerciasis and endemic controls) there was a correlation between the anti-human S-antigen and anti-human IRBP reactivity (Fig. 5; Mann-Whitney rank-sum test; \( P < 0.001 \)).

Discussion

In the experiments described in this article, autoantibodies against human S-antigen and human IRBP were detected in all patients with onchocerciasis and in nearly all the endemic controls. We found no relationship between the levels of antibodies against these retinal proteins and the occurrence of chorioretinitis. These findings indicate that autoimmune reactions against S-antigen and IRBP are not pathognomonic for chorioretinopathy in onchocerciasis. Furthermore, no link was observed between the anti-human...
S-antigen or IRBP antibodies and any ophthalmologic feature of the patients with onchocerciasis.

In this study antibody levels were measured with the use of an ELISA system, whereby the autoantibodies were detected with a peroxidase-conjugated second antibody recognizing the public determinants of the human immunoglobulins. Although variable ratios of different classes of autoantibodies and antibody avidities may have influenced the ELISA reactivity, implicating a poor correlation between ELISA readings and actual autoantibody levels, this method is at present the best quantitative procedure to analyze large series of patient sera.

Autoimmunity against ocular antigens has often been suggested as a possible etiologic factor in the pathogenesis of chorioretinopathy. Evidence for this hypothesis was obtained by Vingtain et al., who investigated the humoral immune response against bovine S-antigen and human retinal extract in onchocerciasis. Autoantibodies against both antigen preparations were found in 11% of patients without retinal involvement, whereas 40% of patients with chorioretinitis had positive results. No difference was found, however, when comparing bovine S-antigen antibody levels between patients with or without chorioretinal involvement, which is in agreement with our findings.

In a later study performed in a larger group of patients, however, the same authors reported a significantly higher level of S-antigen autoantibodies in patients with posterior pole involvement.

Chan et al also demonstrated circulating antibodies against retinal constituents in onchocerciasis using an indirect histochemical immunoperoxidase technique. Of interest was the observation that these autoantibodies could not be absorbed with S-antigen or IRBP, suggesting that other retinal antigens were involved. It is not clear, however, whether the technique used was adequate to pick up circulating antibody activity, which may help to explain the negative absorption studies.

Unlike Vingtain et al., we were not able to detect IgE antibodies against S-antigen in patients with onchocerciasis (data not shown). This was an important observation because in experimental autoimmune uveoretinitis (EAU) induced by bovine S-antigen there is evidence of an IgE-mediated mechanism.

The occurrence of antiretinal antibodies in onchocerciasis may be explained by cross-reactivity of these antibodies with parasitic antigens. We could not demonstrate this because various mAbs against egg antigen or epicuticle antigen of the parasite did not react with human S-antigen or human IRBP (Fig. 4). Moreover, there was no correlation between antibody levels to different stages of parasites or organs of parasites and antiretinal antibody levels. These observations of course do not exclude a possible cross-reactivity. Additional experiments using specifically purified antibodies in various absorption or inhibition studies should be performed to ultimately answer this question.

The finding of retinal autoimmunity in a selected group of people from West Africa could well be explained by the induction of polyclonal B cell activation as a result of parasitic infection. Earlier studies from our group showed that the investigated population from Sierra Leone was infected with at least one to four other parasites and that most people had significantly elevated levels of immunoglobulins compared with Dutch normal donors. Moreover, malaria and trypanosomiasis are endemic in Sierra Leone. In the latter two infections a role for polyclonal B cell activation was clearly demonstrated.

The finding that patients from Surinam and Papua, New Guinea, with Bancroftian filariasis had high levels of antiretinal antibodies is in agreement with the fact that three and, in the latter, four of the mentioned nononchocercal parasitic infections were also endemic in these areas. This explanation of polyclonal activation is supported by the fact that several kinds of autoantibodies have already been demonstrated in patients with onchocerciasis and in endemic control groups. Although the presence of rheumatoid factor (RF) in these sera could have influenced our results, we did not observe an amplification of the signal in the ELISA by adding IgM-RF to our system.

Our conclusion, that antibodies against human S-antigen and human IRBP are not specific for onchocerciasis and are in themselves not sufficient to cause the chorioretinopathy in this disease on the long term, is supported by the observation that EAU could not be induced by passive transfer of antiretinal antibodies. A possible role of antibodies in EAU was indicated by the presence of histologic changes typical of local antigen–antibody reactions and by experiments whereby depletion of complement resulted in a reduced intensity of the disease in guinea pigs. Taken together, these findings suggest that autoantibodies against retinal constituents play an additional role in this primary T-cell–mediated uveitis model. Future studies should be devoted to the question of whether cellular immune reactions against retinal antigens are involved in the chorioretinopathy in onchocerciasis.

Key words: ocular onchocerciasis, chorioretinopathy, autoimmunity, S-antigen, IRBP
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References