Cell-Mediated Immunity Against Human Retinal Extract, S-Antigen, and Interphotoreceptor Retinoid Binding Protein in Onchocercal Chorioretinopathy

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Autoimmune mechanisms are thought to be involved in the pathogenesis of onchocercal chorioretinopathy. Cell-mediated immune responses to human retinal S-antigen, interphotoreceptor retinoid binding protein (IRBP), and crude retinal extract were investigated in patients with onchocerciasis from Sierra Leone, West Africa using a two-step migration-inhibition factor assay. Patients were subdivided into three groups: (1) without ocular involvement (n = 10), (2) with ocular onchocerciasis limited to the anterior segment (n = 19), and (3) with onchocercal chorioretinopathy (n = 21). A group of endemic controls (n = 25) from Sierra Leone were also studied. The cellular immune response to concanavalin A (Con A) was measured to assess the general capacity of lymphocytes to respond to a mitogen. Four of 50 (8%) patients with onchocerciasis and four of 25 (16%) endemic controls reacted with at least one retinal antigen. From the patients with onchocercal chorioretinopathy two of 21 (10%) showed a positive cellular response. The general mitogen response tested with Con A was positive in all these individuals. A role for an antiretinal autoimmune mechanism in the pathogenesis of onchocercal chorioretinopathy, as studied with human S-antigen, IRBP, or crude retinal extract, could not be shown because the cellular response to these antigens did not differ in patients with or without onchocercal chorioretinopathy or in endemic controls. Invest Ophthalmol Vis Sci 31:2031-2036, 1990

Ocular onchocerciasis is a blinding disease. Visual loss can be due to lesions of the anterior segment, eg, sclerosing keratitis and/or lesions of the posterior segment with chorioretinopathy and with optic nerve atrophy. Pathologic changes are often found in both eyes, although the lesions are usually not symmetric and have progressed to different degrees.1

Autoimmunity may play a role in the pathogenesis of this disease. Evidence for this was provided by Vingtain et al,2 who found antibodies against bovine retinal S-antigen and human retinal extract in patients with onchocerciasis from Mali; they claimed there was a significant difference between patients with and without chorioretinal involvement. Our group also investigated the humoral immune response against retinal antigens. Antibodies against human S-antigen and interphotoreceptor retinoid-binding protein (IRBP) were detected in every patient with onchocerciasis and in nearly every endemic control from Sierra Leone, West Africa. No relationship could be found, however, between the level of antibodies against the retinal proteins tested and the occurrence of onchocercal chorioretinopathy.3

The role of cell-mediated immunity against retinal antigens in ocular onchocerciasis is not yet known. In experimental autoimmune uveoretinitis (EAU) induced by a retinal antigen such as S-antigen, it has been shown that the uveoretinitis is primarily T-cell mediated.4-6 This disease could not be elicited by transfer of antiretinal antibodies, although some role for these antibodies cannot be excluded in the disease severity.7

An association of a cellular immune response to human S-antigen with posterior uveitis and panuveitis of mixed etiology was reported by Doekes et al.8 Earlier studies by Nussenblatt et al9,10 showed a proliferative response of lymphocytes to bovine S-antigen in patients with various uveitic entities, including birdshot retinochoroidopathy and, recently, in patients with ocular toxoplasmosis.11

We investigated whether a cell-mediated immune response against retinal antigens. Antibodies against human S-antigen and interphotoreceptor retinoid-binding protein (IRBP) were detected in every patient with onchocerciasis and in nearly every endemic control from Sierra Leone, West Africa. No relationship could be found, however, between the level of antibodies against the retinal proteins tested and the occurrence of onchocercal chorioretinopathy.3

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response against retinal antigens plays a role in the pathogenesis of chorioretinal changes in onchocerciasis. The cellular immune response to retinal antigens was tested by measuring the production of migration inhibition factor (MIF) by peripheral mononuclear cells of patients with onchocerciasis and endemic controls after incubation with human S-antigen, IRBP, or crude retinal extract.

Materials and Methods

Patients and Control Groups

Patients with onchocerciasis (n = 50) and endemic controls (n = 25) tested in this study visited the Eye Hospital Lunsar, which is situated in a hyperendemic area for onchocerciasis in Sierra Leone, West Africa. Onchocerciasis patients were selected on the basis of a positive skin-snip test or a positive Mazzotti test.\(^1\) The mean age in this group was 40.3 yr (range, 12–71 yr), and the female:male ratio was 1:2.6. Individuals with a negative skin-snip test and a negative Mazzotti test were used as endemic controls. Many hospital staff members were among this group. The mean age of the control group was 30.1 yr (range, 13–67 yr) and the female:male ratio, 1:1.1. The patients with onchocerciasis were subdivided in three subgroups: (1) patients without ocular involvement (n = 10), (2) patients with ocular onchocerciasis limited to the anterior segment (n = 19), and (3) patients with onchocercal chorioretinopathy (n = 21). The mean skin-snip count was five to 19 microfilariae for Group 1, 20–49 microfilariae for Group 2, and 50–100 microfilariae per skin-snip for Group 3. None of the patients were being treated with ivermectin, diethylcarbamazine, suramin, or immunosuppressive drugs at that time or during previous 6 months. All subjects were examined according to the Onchocerciasis Survey Form,\(^1\) recommended by the World Health Organization. Chorioretinopathy was scored in cases of early disruption of retinal pigment alone or with exposure of the choroid and clumping of retinal pigment at any degree. Furthermore inflammatory activity in the posterior pole was noted.

Venous blood was drawn from patients and endemic controls using Venoject evacuated blood collecting tubes with heparin (Terumo Europe, Leuven, Belgium). Peripheral blood mononuclear cells (PBMC) were isolated from this heparinized blood by centrifugation on Ficoll-Paque (Pharmacia, Uppsala, Sweden), washed twice with phosphate-buffered saline (PBS), and used immediately in the first step of the MIF assay.

Informed consent from patients and controls was obtained after explanation of the study.

Human S-antigen, IRBP, and Crude Retinal Extract

The retinal antigens were isolated from retinas of white human donor eyes from which the cornea had been removed for transplantation. These retinas were stored at −20°C until use.

The isolation of human S-antigen was done as described previously.\(^2\) Briefly a 50% ammonium sulfate precipitation of retinal extract was followed by DEAE anion-exchange chromatography and gel filtration.

Human IRBP was purified using a modification of the method described by Adler et al.\(^12,13\) After thawing, the retinas were extracted with cold PBS containing 0.1 mM phenylmethyl-sulfonyl fluoride (PMSF; Sigma, St. Louis, MO) and 0.02% NaN\(_3\), pH 7.4, for 1 hr at 4°C. After centrifugation for 30 min at 3000 \(\times\) 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 Amicon XM-50 filter (Amicon, Danvers, MA). Further isolation was done by 30% ammonium sulfate precipitation and affinity chromatography on a concanavalin A (Con A)-Sepharose column (Pharmacia) in PBS, containing 0.5 M NaCl, 0.2 mM PMSF, and 0.02% (w/v) NaN\(_3\), (pH 7.4). Human IRBP was eluted with 2.0 M methyl \(\alpha\)-D glucopyranoside (Sigma) in the same buffer. Finally, gel filtration on a Sephacryl-S300 column (Pharmacia) was done in the same buffer as that used for the Con-A-Sepharose column, with the addition of 0.1% (v/v) Tween 20. At every step of the isolation procedure, human IRBP was detected with sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and an enzyme-linked immunosorbent assay using a rabbit anti-bovine IRBP antiserum (kindly provided by Dr. R. M. Broekhuysen, Nijmegen, The Netherlands).

The homogeneity and the identity of human S-antigen and human IRBP were confirmed by SDS-PAGE, using samples of approximately 10 \(\mu\)g each, with Coomassie Brilliant Blue (Merck-Schuchardt, Darmstadt, FRG) staining of the gels. Both proteins showed one band on the gels. The molecular weight was 50 kilodaltons (kD) and 135 kD, respectively (data not shown).

The crude retinal extract was prepared by the same extraction procedure as originally with human IRBP, but instead of 1 hr, the extraction proceeded for 20 hr at 4°C. After centrifugation for 30 min at 3000 \(\times\) g, the supernatant was collected.

Protein concentrations were determined with the Bradford-method,\(^14\) using bovine serum albumin (Sigma) as a standard.

The S-antigen, IRBP, and the crude retinal extract were dialyzed and, if necessary diluted, in

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RPMA-1640 supplemented with 25 nM HEPES, 100 units/ml of penicillin, and 0.1 mg/ml of streptomycin (Gibco, Grand Island, NY).

MIF Assay

The cellular immune response against human S-antigen, IRBP, or crude retinal extract was measured in a MIF assay. The test consisted of two parts. In the first step, done in the Eye Hospital Lunsar, Sierra Leone, the PBMC were incubated with the test antigens. The cell supernatants were collected, frozen, and subsequently tested in the second step for the presence of MIF by using the human monocytoid U937 cell-line as indicator cells. This second step was done in our laboratory in Amsterdam, The Netherlands as described by Thurman et al.15

Briefly, the isolated PBMC were adjusted to 2.5 × 10^6 cells/ml in RPMI-1640 supplemented with 25 nM HEPES, 100 units/ml of penicillin, 0.1 mg/ml of streptomycin (Gibco), and 10% heat-inactivated pooled human serum from nontransfused male white donors. One-mL aliquots of the cells were dispensed into five separate 10-ml culture tubes (Nunc, Roskilde, Denmark). One tube served as a medium control for spontaneous MIF production of the cells; the second tube was used to test the overall ability of the cells to respond to adding Con A (in a final concentration of 25 µg/ml). In three other tubes human S-antigen (5 µg/ml), IRBP (10 µg/ml), and crude retinal extract (103 µg/ml) were added. A sixth tube contained culture medium alone without any cells. The tubes were incubated for 18 hr at 37°C. Supernatants were harvested by centrifugation (10 min at 1200 X g) and stored at -20°C until the second step of the assay.

For the second step of the assay, U937 cells were harvested from permanent in vitro cultures, washed, and adjusted to 500 × 10^6/ml in culture medium. Seaplaque agarose (FMC Corporation, Rockland, ME) was dissolved (22.5 mg/ml) in PBS at 100°C for 20 min and diluted 1:10 with warm (37°C) culture medium. Equal volumes of this agarose solution and U937 cells were mixed and kept at 37°C. After storage of the tray for 15 min at 4°C, 0.1 ml of the cell-culture supernatants were added fourfold. The tray was incubated for 20 hr in a humidified incubator at 37°C and 5% CO₂ to allow migration of the U937 cells out of the agarose droplets.

The migration areas were measured by planimetry and the migration inhibition index (MI) was calculated as follows:

\[
MI = \frac{\text{mean area test supernatant}}{\text{mean area control supernatant}}
\]

in which the control supernatant came from the tube containing cells in culture medium without antigen. A MI < 0.8 was taken as a positive reaction.

Results

To investigate whether the cellular immune response against retinal antigens plays a possible role in the pathogenesis of the chorioretinopathy in onchocerciasis, PBMC of onchocerciasis patients and endemic controls were challenged with human S-antigen, IRBP, or crude retinal extract.

No difference could be shown between patients with or without onchocerocal chorioretinopathy and endemic controls with regard to their ability to react in vitro human S-antigen, IRBP, or crude retinal extract (Fig. 1). The general mitogenic responsiveness of West Africans when tested with Con A was positive.

Lymphocytes from four of 50 (8%) patients with onchocerciasis showed MIF production (MI < 0.80) after overnight incubation with at least one of the retinal preparations (Table 1). One patient without ocular involvement had a positive response to human S-antigen (one of ten [10%]) and did not react with human IRBP or crude retinal extract. A second patient with ocular onchocerciasis, but without chorioretinal changes, showed a MI < 0.80 in response to both human S-antigen and IRBP (one of 19 [5%]) but not to crude retinal extract. Of the remaining two patients with onchocerocal chorioretinopathy, one patient responded to human S-antigen, IRBP, and crude retinal extract. The other patient's PBMC reacted only to human S-antigen and IRBP (two of 21 [10%] and one of 21 [5%], respectively) (Fig. 1; Table 2).

In the group of endemic controls four of 25 (16%) positive reactions were observed after challenge of their cells with at least two of the three retinal proteins (Table 1). Two of them showed MIF production with human S-antigen, IRBP, and crude retinal extract, and the other two only reacted to S-antigen and IRBP (Fig. 1; Table 2).

The general capacity of the PBMC of the patients and controls to respond in vitro was tested by incubating the cells with the mitogen Con A. All individuals showed a strong positive response, when tested with Con A (Fig. 1). The mean MI in the four tested groups varied between 0.24–0.31 (with standard deviations varying between 0.08–0.14).
Discussion

For several decades autoimmune mechanisms have been thought to play a role in the pathogenesis of the chorioretinal changes in ocular onchocerciasis. In our study no relationship could be established between a cellular immune responsiveness to human S-antigen, IRBP, or crude retinal extract and the occurrence of chorioretinopathy in onchocerciasis. Only two of 21 patients (10%) with onchocercal chorioretinal lesions showed a reaction to at least two of these retinal proteins, whereas four of 25 individuals (16%) of the endemic control group reacted positively. There is evidence implicating inflammation, mediated primarily by T-cells, in EAU, although a role has been claimed for a humoral immune response against retinal antigens in this model. In addition to this, several investigators confirmed the observation that an association exists between some form of clinical uveitis and cell-mediated immunity against retinal antigens.

There are no reports to our knowledge on cell-mediated autoimmunity against retinal antigens in ocular onchocerciasis. Only defects in cellular immunity have been described with preservation of reactivity to mitogens in onchocerciasis. Diminished cell-mediated immunity to parasite-related and nonparasite-

Table 1. Cellular immunity against retinal antigens in Sierra Leone (West Africa)

<table>
<thead>
<tr>
<th></th>
<th>No. (percentage) of individuals* with a migration inhibition index &lt; 0.80</th>
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<tbody>
<tr>
<td></td>
<td>n</td>
</tr>
<tr>
<td>Onchocerciasis patients</td>
<td>50</td>
</tr>
<tr>
<td>Without ocular involvement</td>
<td>10</td>
</tr>
<tr>
<td>With ocular involvement</td>
<td></td>
</tr>
<tr>
<td>Limited to the anterior segment</td>
<td>19</td>
</tr>
<tr>
<td>Chorioretinopathy</td>
<td>21</td>
</tr>
<tr>
<td>Endemic control subjects</td>
<td>25</td>
</tr>
</tbody>
</table>

* See table 2 for specific response to each antigen in separate individuals.
related antigens was reported. It is possible, that this results in a protective mechanism avoiding tissue damage due to reaction against dead microfilariae in this parasitic infection.

We measured the cellular immune response to human S-antigen, IRBP, and crude retinal extract in patients with onchocerciasis with and without chorioretinal involvement and in endemic controls from Sierra Leone, using a two-step MIF-assay. Type IV (Gell and Coombs) allergic reactions, presumed to play a role in cellular autoimmunity, are often investigated using the lymphocyte transformation test. A better and more consistent correlation exists, however, between the MIF assay and the delayed-type hypersensitivity skin reaction.

Neither cell-mediated nor humoral autoimmunity against retinal antigens such as human S-antigen, IRBP, and crude retinal extract could specifically be shown in patients with ocular onchocerciasis and, in particular, in patients with onchocercal chorioretinopathy compared with the endemic controls. We are inclined to return to an earlier concept in which microfilariae of *Onchocerca volvulus* or their toxins are directly involved in the pathogenesis of the chorioretinal changes in onchocerciasis. Histologic studies report microfilariae in the retina and choroid, and Murphy et al described microfilariae in the living retina examined with a three-mirror contact lens at the biomicroscope. There is also the observation that posterior-segment lesions can freshly develop or progress after treatment with diethylcarbamazine, probably by killing the microfilariae in situ, which gives further support to this hypothesis.

**Key words:** onchocerciasis, retinal autoimmunity, cell-mediated immunity, S-antigen, IRBP

**Table 2. Patients and control subjects with a positive cell-mediated immune response to retinal proteins**

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age</th>
<th>Sex</th>
<th>MF per skin snip</th>
<th>S-antigen</th>
<th>IRBP</th>
<th>Retinal extract</th>
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<tr>
<td>Onchocerciasis patients</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Without ocular involvement</td>
<td>55</td>
<td>22</td>
<td>F</td>
<td>1-4</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>With ocular involvement</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Limited to the anterior segment</td>
<td>20</td>
<td>40</td>
<td>M</td>
<td>1-4</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Chorioretinopathy</td>
<td>46</td>
<td>45</td>
<td>F</td>
<td>&gt;100</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>53</td>
<td>35</td>
<td>M</td>
<td>1-4</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Endemic control subjects</td>
<td>22</td>
<td>19</td>
<td>F</td>
<td>0</td>
<td>+</td>
<td>−</td>
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<td>71</td>
<td>20</td>
<td>F</td>
<td>0</td>
<td>+</td>
<td>+</td>
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</table>

* All individuals, including patients and control subjects who did not react to human S-antigen, IRBP, or retinal extract, had a positive cellular response to Con A. MF = microfilariae.

**References**


