Immunity to a Corneal Antigen in Fuchs' Heterochromic Cyclitis Patients

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Immunity to a major corneal antigen was studied in 28 Fuchs' heterochromic cyclitis patients and compared with the response of 44 patients with other types of uveitis and 19 healthy controls. The highest incidence of immune response was found in patients with anterior segment involvement only (anterior uveitis and Fuchs') whereas the frequency of anti-corneal immune response in patients with posterior segment involvement only was low and not significantly different from that of healthy controls. Cellular immunity to corneal antigens was found in the majority of Fuchs' heterochromic cyclitis patients, and in one-third of the anterior uveitis patients. No correlation could be established in these patients between a positive cellular response and the chronicity of the disease or the presence of keratic precipitates. Humoral immunity to the corneal antigen was also the highest in patients with anterior segment involvement, but there was no difference in response between Fuchs' and non-Fuchs' anterior uveitis patients. This study suggests that anti-corneal immunity may be triggered in inflammatory diseases of the anterior segment, especially in Fuchs' heterochromic cyclitis.

Fuchs' heterochromic cyclitis is an infrequent, mostly unilateral uveitis. It usually affects the iris stroma first, resulting in a variable degree of atrophy and causing depigmentation. Small white or translucent keratic precipitates are characteristic of the disease. These keratic precipitates are not concentrated solely in the inferior triangle of the cornea, as they are in the other uveitis entities, but are evenly and widely scattered over the entire corneal endothelium.

The pathogenesis of Fuchs' heterochromic cyclitis is essentially unknown. Histopathological analysis of the rare specimens available has shown extensive numbers of plasma cells in the iris stroma, on the surface of the iris and in the trabecular meshwork. Fine fibrillar material was also seen deposited on the posterior corneal surface. Many newly formed vessels were seen on the surface of the iris. The keratic precipitates on the corneal endothelium are deposits of leukocytes, chiefly monocytes and lymphocytes. Dernouchamps, in a review paper, reported high levels of immunoglobulins, mainly IgG, and immune complexes in the aqueous humor of Fuchs' heterochromic cyclitis patients. The author suggested that these higher levels of immunoglobulins may be the result of an immune response to infectious agents or autoantigens.

Earlier investigations have already demonstrated that uveitis patients and Fuchs' heterochromic cyclitis patients, in particular, have circulating antibodies to corneal antigens. In the study reported here we have investigated whether cellular and humoral immunity to one of the major soluble corneal antigens is different in Fuchs' heterochromic cyclitis patients when compared to patients with other types of uveitis. Our results show that immunity to the 54 kD corneal protein is found with the highest frequency in Fuchs' heterochromic cyclitis patients.

Materials and Methods

Patient and Control Population

Heparinized and clotted blood samples were obtained from 72 uveitis patients attending the Eye Clinic in Rotterdam (Dr. Baarsma) or the Academic Medical Centre in Amsterdam (Dr. Rothova). At the same time similar samples were obtained from healthy laboratory staff to serve as controls (n = 19, 14 males and 5 females; mean age 33 years, range 26 to 57). All patients and controls were informed about these investigations and their consent was obtained. The uveitis patients were subdivided according to the
location and type of their uveitis into: anterior uveitis, Fuchs' heterochromic cyclitis, panuveitis and posterior uveitis (Table 1).

**Isolation of the 54 kD Corneal Antigen**

The isolation procedures have been described extensively elsewhere. Bovine eyes, obtained from the local slaughterhouse, were used to extract the antigen. Previously we demonstrated that circulating anti-corneal antibodies reacted similarly with corneas from different species. Bovine corneas were used since they are easily obtainable and provide large quantities of material. In brief, the isolation involves the scraping of corneal epithelium, homogenization and extraction for 48 hr in a 3 M KCl 0.1 M EDTA phosphate buffer, pH 7.4. The 54 kD corneal antigen was subsequently purified by anion exchange chromatography and showed only one band when tested by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) using a Coomassie Brilliant Blue R250 (Merck, Darmstadt, W. Germany) staining.

**Migration Inhibitory Factor (MIF) Assay**

MIF production was tested using a two-step migration inhibition assay. For the first step mononuclear cells were isolated from the heparinized blood using density gradient centrifugation on Ficoll-Paque (Pharmacia, Uppsala, Sweden). Cells from one or more controls were always tested on the same day as the patient cells. The cells were washed twice and adjusted to 2.5 X 10^6 cells per ml in culture medium. Seaplaque agarose (FMC Corporation, Rockland, ME) was dissolved in phosphate-buffered saline (PBS) at 100°C for 20 min and subsequently diluted ten-fold with warm (37°C) culture medium. Equal volumes of the agarose solution were mixed with the U937 cell suspension and kept at 37°C. One microliter droplets were pipetted with a 50 μl Hamilton syringe, fitted with a mechanic dispensing device, into wells of a flat-bottomed 96-well microtiter tray (Nunc, Roskilde, Denmark). The droplets were allowed to set for 30 min at 4°C and 100 μl of cell-free supernatant, obtained in the first step, was added to each well. Each supernatant was tested in five-fold. The trays were incubated for 20 hr in a humidified incubator at 37°C and 5% CO₂. The areas of monocyte migration were measured and the migration indices (MI), calculated as follows:

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

The control supernatants came from tubes containing mononuclear cells in culture medium only. These tubes also served to check for spontaneous MIF production, since the areas of monocyte migration in these supernatants should not differ markedly (<20%) from those obtained in culture medium which has never been in contact with mononuclear cells. In these experiments the differences between these two control migration areas varied from 0 to 10%. Other controls included the addition of the 54 kD corneal antigen or of con-A to the monocyte droplet; no influence on monocyte migration was seen. The reproducibility of the test was checked with some of the patients and the controls. Cells were obtained twice in 1 week and after 1-week intervals, on all occasions a concordant response was seen.

A mean migration index of 0.95 ± 0.07 was calculated from the control values, and subsequently MI values equal to or smaller than 0.80 were taken as positive reactions.

**ELISA Test for the 54 kD Corneal Antigen**

Serum was isolated from the clotted blood and stored in small portions at −20°C until used for the ELISA. Polystyrene microcuvettes (Gilford, Cleveland, OH) were coated with 300 μl purified corneal antigen (5 μg/ml) in phosphate-buffered saline (PBS). The microcuvettes were incubated at room temperature on a shaker for 2 hr, washed twice with PBS containing 0.1% Tween 20 (PBS-Tween, Brocacef, Maarsen, The Netherlands). Next, 300 μl of patient or control serum, diluted 1:50 in PBS-Tween, was added. After 1 hr incubation at 37°C the cuvettes were thoroughly washed and allowed to drain inverted on a paper towel for 1 min. After incubation with 300 μl peroxidase conjugated goat anti-human...
immunoglobulin (Nordic, Tilburg, The Netherlands) diluted 1:2000 in PBS-Tween for 1 hr and washing, binding of antibodies to the corneal antigen was made visible by adding 350 μl ABTS (0.16 mM 2.2 azino - di - 3 - ethyl - benzthiazoline - 6 - sulphonate [Boehringer, Mannheim, West Germany] and 0.15% H2O2 in 0.05 M citric acid, pH 4.0) solution at room temperature. The green reaction product was measured after 20 min in a spectrophotometer (EIA, Gilford) at 405 nm. Controls included uncoated cuvettes and coated cuvettes incubated with PBS-Tween only.

Using a strong positive serum in different dilutions (1/50, 1/100, 1/200, 1/400) as a day-to-day standard, ELISA units were calculated for each serum. The OD (405 nm) values obtained with the dilutions of the standard serum were set out in a graph, the 1/50 dilution being set arbitrarily at 100 units. The ELISA units for each patient were calculated from the OD (405 nm) values obtained for their serum.

In the control group 13 out of 14 sera had 35 ELISA units or less; hence, 36 ELISA units was chosen as the cut-off point for positive or negative results. In this case it was not possible to calculate the upper limit of the normal range using a mean value plus two standard deviations since the one positive result was expressed as >100 units. The mean ± SD of the sera from the 13 controls taken as negative was calculated to be 22.0 ± 6.8, so the cut-off point of 36 ELISA units was chosen.

Statistics

Statistical analysis of the data was performed using the Fishers exact test or the correlation coefficient.

Results

Cellular Immunity to the 54 kD Corneal Antigen

The MIF assay detects the presence of lymphokines produced in vitro, in response to an antigen, by lymphocytes sensitized in vivo to the same antigen. A good correlation of the MIF assay with delayed hypersensitivity in vivo has been found. A positive reaction in the MIF assay with the 54 kD corneal antigen was found in one of the 19 healthy controls (5%). This person admitted having had trauma to the cornea after being hit with a hockey ball a few years ago; the humoral response, however, was negative. The other controls had no history of corneal lesions, although some were contact lens wearers.

In uveitis patients an increase of responsiveness to the 54 kD corneal antigen was found. Patients with posterior segment involvement only had a markedly lower incidence of response than those with anterior segment involvement. 22.0 ± 6.8

Table 1. Clinical characteristics of the uveitis patients

<table>
<thead>
<tr>
<th></th>
<th>Anterior uveitis</th>
<th>Fuchs' uveitis</th>
<th>Posterior uveitis</th>
<th>Pan uveitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>18</td>
<td>28</td>
<td>14</td>
<td>12</td>
</tr>
<tr>
<td>Male:female</td>
<td>1:1</td>
<td>1:1.25</td>
<td>1:1.3</td>
<td>1:0.7</td>
</tr>
<tr>
<td>Mean age (years)</td>
<td>40</td>
<td>29</td>
<td>32</td>
<td>40</td>
</tr>
<tr>
<td>Age range</td>
<td>(23-63)</td>
<td>(12-71)</td>
<td>(12-55)</td>
<td>(18-72)</td>
</tr>
<tr>
<td>Etiology unknown</td>
<td>11</td>
<td>4</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Sarcoidosis</td>
<td>1</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Toxoplasmosis</td>
<td>7</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parsplantis</td>
<td>2</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HLA B27+</td>
<td>4*</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>2</td>
<td>28</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

* Two patients had ankylosing spondylitis.

only two (14%) of the uveitis posterior patients had sensitized lymphocytes against the 54 kD corneal antigen; these two patients were diagnosed as having toxoplasma uveitis. The incidence of positive responses increases when the anterior segment of the eye is involved; three (25%) of the panuveitis patients, six (42%) of the anterior uveitis patients and 20 (71%) of the patients with Fuchs' heterochromic cyclitis had a positive cellular immune reaction to the 54 kD corneal antigen. To determine the specificity of this cellular response, the uveitis patients and the controls were also tested for their cellular response to one other ocular antigen. The response to human S-antigen was performed as described by Doekes et al, and no correlation (r = 0.1) was found between the cellular immune response to S-antigen and to the 54 kD corneal antigen.

Fuchs heterochromic cyclitis is characterized by a chronic low-grade inflammation and the presence of keratic precipitates. We therefore sought to establish a relationship between the chronicity of the disease or...
the presence of keratic precipitates and the response to the 54 kD corneal antigen in the anterior uveitis patients (Fuchs' and non-Fuchs'). No relation was seen between a positive cellular response to the 54 kD corneal antigen and the chronicity of the disease ($P = 0.09$) or the presence of keratic precipitates ($P = 0.26$). Within the group of chronic anterior uveitis patients there was no significant difference in response to the 54 kD corneal antigen ($P = 0.25$) between Fuchs' and non-Fuchs' patients.

**Humoral Immunity against the 54 kD Corneal Antigen**

Using the immunofluorescence test, serum antibodies to corneal epithelium antigens were found in uveitis patients, but this assay does not permit discrimination between the different antigens involved. When purified antigens are available, the ELISA may be used to study the response to one defined antigen at the time.

Sera from 60 of the 72 patients and from 14 of the 19 controls were available for testing for the presence of circulating antibodies against the 54 kD corneal antigen using an ELISA. Figure 2 shows the antibody levels, expressed in units, in the various groups. One control had high levels of circulating antibody to the 54 kD corneal antigen, but no cellular immunity. Neither the medical history nor ophthalmological examination disclosed any corneal abnormalities in this person.

Two (17%) posterior uveitis patients and two (25%) panuveitis patients had antibodies to the 54 kD corneal antigen. The etiology was unknown in the two panuveitis patients. The two posterior uveitis patients with antibodies to the 54 kD corneal antigen were diagnosed as having toxoplasma uveitis, but they were not the same two patients who had cellular immunity to the 54 kD corneal antigen (Fig. 3). Of the anterior uveitis patients, eight (53%), and of the Fuchs' heterochromic cyclitis patients, 12 (48%) had antibodies to the 54 kD corneal antigen. In the eight non-Fuchs' anterior uveitis patients the etiology was unknown in two cases, sarcoidosis in two cases, syphilis in two cases and two cases were HLA B27-positive, one of these patients having ankylosing spondylitis.

The overall incidence of circulating antibodies to the 54 kD corneal antigen in the total group of uveitis patients was 34% (Fuchs' heterochromic cyclitis patients excluded since they are over-represented in this study compared to the random uveitis population), which corresponds well with the 27% reported in an earlier study.11

**Relationship between Cellular and Humoral anti-54 kD Corneal Antigen Response**

Both in the uveitis patients and in the healthy controls, no positive or negative correlation was found between cellular and humoral immunity to the 54 kD corneal antigen (Table 2). In the controls no person was seen to have both cellular and humoral immunity to the 54 kD corneal antigen. When the uveitis patients were subdivided according to their diagnosis, it appeared that in Fuchs' heterochromic cyclitis patients one-third made both types of immune response, whereas in anterior and panuveitis it was only one-eighth, and in posterior uveitis, none (Fig. 3).

Within the group of patients with anterior segment involvement a significant difference ($P < 0.05$) was found in cellular immunity, but no significant difference in the humoral response ($P > 0.5$) between non-Fuchs anterior uveitis and Fuchs' heterochromic cyclitis patients.

**Discussion**

Our results show a high incidence of immune response to the 54 kD soluble corneal antigen in uveitis patients with anterior segment involvement. Cellular immunity to this corneal antigen was most frequently found in Fuchs' heterochromic cyclitis patients, whereas humoral immunity was found in about half (range 25 to 53%) of all the patients with anterior segment involvement, but only in 17% of the patients with posterior segment involvement. This study extends a previous one by La Hey et al.,10 where antibodies to corneal epithelium were demonstrated in 88% of the patients with Fuchs' heterochromic cyclitis using the indirect immunofluorescence assay on whole cornea sections. In that study
Fig. 3. Distribution of the different types of immune response to the 54 kD corneal antigen found in the various uveitis entities. In the controls (not shown) the distribution was similar to that found in posterior uveitis patients: 86% no response, 7% cellular immunity only and 7% humoral immunity only.

Table 2. Cellular versus humoral immunity to the 54 kD corneal antigen in uveitis patients and healthy controls

<table>
<thead>
<tr>
<th>Humoral immunity</th>
<th>Uveitis patients</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellular immunity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>20</td>
<td>12</td>
</tr>
<tr>
<td>Positive</td>
<td>13</td>
<td>0</td>
</tr>
</tbody>
</table>
|                  | *P = 0.21*      | *P = 0.93*

No circulating antibodies to iris tissue could be detected, although iris tissue is reported to be the first affected in Fuchs' heterochromic cyclitis. In the current study we investigated the immune response to one isolated corneal antigen, the 54 kD corneal antigen. This antigen accounts for 30% of the soluble corneal proteins and is present, or cross-reactive with, antigens in the lens epithelium and the ciliary body.16

Humoral immunity to this 54 kD corneal antigen was found in 48% of the Fuchs' heterochromic cyclitis patients. When the results of the previous study15 and of this investigation are compared, it is apparent that some of the anti-corneal antibodies found with the immunofluorescence test (88% positive response) in Fuchs' heterochromic cyclitis patients must be directed at antigens other than the 54 kD corneal antigen. These may be other soluble corneal antigens, but the non-anti-54 kD antibodies may also be directed towards insoluble corneal proteins. In the current study no aqueous humor taps were obtained from these patients, and therefore it was not possible to correlate the systemic antibody response with possible local antibody production. In the non-Fuchs' anterior uveitis patients it was striking to see that in 75% of the patients with antibodies to the 54 kD corneal antigen the underlying etiology was also known.

Cellular immunity to the 54 kD corneal antigen was by far the most pronounced in the Fuchs' heterochromic cyclitis patients (71% positive response) and was found in only two of the posterior uveitis patients (14%). Although it is not yet clear whether the typical keratic precipitates seen on the corneal endothelium of Fuchs' heterochromic cyclitis patients are due to specifically sensitized lymphocytes, it is possible that such sensitized cells leave the blood vessels, when their permeability is increased during inflammation, and find their target antigen in the anterior chamber. Experimental studies in rabbits have shown that injection of lymphocytes, specifically sensitized to the allogeneic antigens of the recipient, into the anterior chamber of the eye results in focal pock formation on and localized destruction of the corneal endothelium.17 The pocks on the corneal endothelium in this experimental model histologically mimic the keratic precipitates observed in Fuchs' heterochromic cyclitis patients. It is therefore suggestive to think that the inflammatory cells in the keratic precipitates of Fuchs' heterochromic cyclitis patients might be specifically sensitized to corneal endothelium antigens. Studies with anti-54 kD corneal antigen-sensitized cells or anti-54 kD antibodies injected into the ante-
ior chamber of an animal will have to be performed to demonstrate whether this hypothesis is true.

From the differences in type of immune response found in Fuchs' heterochromic cyclitis and other anterior uveitis entities, the assumption could be made that the manner of antigen presentation might be different in these diseases. From the study of anterior chamber-associated immune deviation (ACAID) it appeared that antigen presented via the anterior chamber gave rise to systemic humoral immunity soon after inoculation, whereas the cellular immune response was suppressed. The same antigens presented via the intravenous route or via the footpad gave rise to both cellular and humoral immunity, measurable 7 days after inoculation.

The role of the anti-corneal immune response found in uveitis patients with anterior segment involvement is still not elucidated. Until an immunopathological effect has been demonstrated, the immune response may be considered secondary to leakage of normally sequestered antigens and the differences found in various uveitis entities may be due to possible differences in presentation of this antigen. Long-term studies of Fuchs' heterochromic cyclitis patients, both clinically and immunologically, should determine whether this anti-54 kD corneal antigen immune response has any predictive value with regard to the long-term outcome of the disease, or whether it should be regarded as an epiphenomenon.

Key words: Fuchs' heterochromic cyclitis, uveitis, corneal antigen, migration inhibition factor, autoimmunity

References