Serum antibody levels of uveitis patients to bovine retinal antigens

Dale S. Gregerson, I. Willard Abrahams, and Charles E. Thirkill

Serum samples from 91 uveitis patients and controls were tested by the enzyme-linked immunosorbent assay technique to determine their relative antibody titers to bovine retinal S antigen and to a detergent extract of saline-washed bovine retina, or P antigen. Only those patients whose uveitis fell into the categories of toxoplasmosis, iritis, herpes keratouveitis, pars planitis, sarcoidosis, Behçet's syndrome, and Vogt-Koyanagi-Harada syndrome are presented in this study. Significantly elevated titers (p < 0.05) of antibody to S antigen were found in the diagnostic categories of iritis, toxoplasmosis, sarcoidosis, and Behçet's. Elevated titers to the detergent extract were found in iritis (granulomatous and hypopyon subcategories only), toxoplasmosis, sarcoidosis, and Vogt-Koyanagi-Harada syndrome. The anti-S titers of patients treated with systemic steroids were depressed below normal levels. A separate control group of patients with nonuveal ocular inflammations (conjunctivitis, episcleritis, etc.) had titers very similar to normal controls. The posttreatment titers to S antigen of patients receiving laser photocoagulation for diabetic retinopathy were significantly elevated over normal controls. (INVEST OPHTHALMOL VIS SCI 21:669-680, 1981.)

Key words: uveitis, retinal antigens, autoimmunity, enzyme-linked immunosorbent assay (ELISA), chorioretinitis, diabetic retinopathy, laser photocoagulation

Autoimmune responses to retinal antigens have been postulated to be causes or contributing factors in some kinds of uveitis. Supporting evidence for this hypothesis has been found primarily in animal models of experimental allergic uveitis (EAU), in which a uveitis-like disease can be induced in several species of laboratory animals, including guinea pigs, rats, and primates, by immunizations in remote sites with various ocular tissues,1-4 retinal rod outer segments (ROS),5,6 S antigen,7,8 and rhodopsin9 in adjuvant.

Testing for immune reactivity to these antigens in humans has been hindered by several factors, including a shortage of antigen, low titers of specific antibody, frequent use and need for immunosuppressive (i.e., steroid) therapy, relatively small numbers of patients, the need for a highly sensitive assay, and the fact that many uveitis patients cannot be readily categorized with respect to their clinical signs for the purposes of a study.

Recently the lymphocyte transformation assay has been used to assess the cell-mediated immune response to human and bovine S antigen in various types of human uveitis.10 Wide variations in reactivity were observed, but stimulation indices greater than 2 were found in some patients, particularly those with toxoplasmosis and sarcoidosis.

From the Department of Ophthalmology and Visual Sciences, Yale University School of Medicine, New Haven, Conn.

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Reprint requests: Dale S. Gregerson, Ph.D., Yale University School of Medicine, Department of Ophthalmology and Visual Sciences, 333 Cedar St., BML B219, New Haven, Conn. 06510.
Data on cell-mediated immunity to either P antigen or purified rhodopsin (or opsin) in uveitis patients has not been reported at this time. The presence of antiuveal antibodies in uveitis patients has been reported, although the antigens used in the assays were not identified or characterized. The presence of autoreactivity to soluble and particulate retinal antigens has also been reported in cases of retinitis pigmentosa.

In this study we have measured the serum antibody levels of uveitis patients and several different types of controls to bovine S antigen and partially purified bovine P antigen in an indirect enzyme-linked immunosorbent assay (ELISA). We have attempted to clarify the roles and contributions of the various retinal antigens in uveitis by utilizing a variety of controls. Thus normals, patients with non-uveal ocular inflammations, steroid-treated patients, and photocoagulated diabetic patients have been used in this study. Patients being laser-treated for diabetic retinopathy were chosen to test the hypothesis that retinal destruction alone is sufficient to stimulate an antiretinal immune response. To our knowledge this is the first report of an elevated antiretinal immune response in laser-treated diabetic patients.

Materials and methods

Patients. The antibody titers reported here were obtained from blood samples taken from 91 uveitis patients of seven major diagnostic categories as well as from normal controls, prelaser and postlaser diabetic patients, and other controls. With the exception of the Behçet's and Vogt-Koyanagi-Harada (VKH) blood samples obtained from Dr. Kanshiro Masuda, Department of Ophthalmology, Tokyo University, Japan, and the prelaser and postlaser diabetic blood samples kindly provided by Dr. James Puklin, Department of Ophthalmology and Visual Sciences, Yale University, all other specimens were obtained from patients of the Uveitis Service at Yale University or from private patients of I. W. A. All the uveitis patients were examined and followed by I. W. A. Blood specimens were drawn from patients at the initial examination, and serial samples were taken at subsequent visits. Serial samples were not obtained from patients receiving systemic steroids, except as a control for the effect of steroids on the immune responses.

The criteria used for establishing a specific clinical diagnosis were those in common usage, as follows:

1. **Iritis-nongranulomatous.** Anterior segment inflammation manifested by ray, cells, circumlimbal injection, with no focal posterior involvement, no mutton-fat keratic precipitates (KP), no pars plana or retinal involvement, and no laboratory data suggestive of other more specific diagnoses (toxoplasmin indirect fluorescein antibody titer, fluorescent treponemal antibody-absorption (FTA-ABS, etc.).

2. **Iritis-granulomatous.** Presence of ray, cells, mutton-fat KP, but no posterior lesions, no positive specific etiologic immunologic tests (toxoplasmin indirect fluorescein antibody titer, etc.) or evidence of systemic disease (sarcoid, syphilis, etc.), and negative angiotensin-converting enzyme (ACE).

3. **Hypopyon uveitis.** Anterior segment involvement with presence of hypopyon on at least one occasion without posterior involvement, without mutton-fat KP, absence of endophthalmitis, and no other systemic involvement or positive laboratory data.

4. **Fuchs' heterochromic iridocyclitis.** Presence of unilateral iritis, heterochromia, moth-eaten pigment epithelium, cataractous changes in the lens, with no other retinal or systemic involvement or positive laboratory data.

5. **Pars planitis.** Cells in the retrorenal space, exude over the pars plana area, far peripheral retinal vascular inflammation, no focal posterior lesions or other positive laboratory data.

6. **Sarcoïd.** Clinical picture compatible with ocular sarcoid, i.e., perivasculitis with candlewax drippings, heavy mutton-fat KP, preretinal nodules, broad-based peripheral anterior synchia in the chamber angle, with hilar adenopathy diagnosed by the Pulmonary Service as sarcoïdosis, positive ACE, gallium scan, etc.

7. **Toxoplasmosis.** Focal retinochoroiditis plus vitreitis compatible with clinical picture of ocular toxoplasmosis with characteristic evolution of lesions along with positive toxoplasmin indirect fluorescein antibody test.

Each uveitis patient had a complete medical, social, and eye history, complete eye examination, including best corrected vision, external examination, slit-lamp examination, gonioscopy, indirect
ophthalmoscopy, tensions, peripheral fields, fundus photography and angiography when possible, and Amsler fields when applicable. Venereal Disease Research Laboratory (VDRL) titers, FTA-ABS, toxoplasmin indirect fluorescein antibody titers, histoplasmic complement fixation tests, and *Ascaris* and *Toxocara* ELISA tests were performed, and chest films were obtained. Special tests, i.e., ACE and gallium scan, and consultations pertaining to pediatric, pulmonary, neurologic, gynecologic, and dental backgrounds were obtained as indicated, and all prior records were reviewed.

**Preparation of serum samples.** Serum was drawn off 10 ml of clotted blood, clarified by low-speed centrifugation, heat-inactivated (56° for 30 min), and stored at -20°. In a few cases the blood was heparinized and spun at low speed to separate the plasma, which was heat-inactivated, centrifuged at 10,000 × g, and stored at -20°. Serum samples of guinea pig anti–guinea pig S antigen (GPaGP-S) and guinea pig anti–guinea pig ROS (GPaGP-ROS) were kindly provided by Dr. Waldon Wacker. These sera are mainly directed against S antigen and rhodopsin, respectively. Rabbit anti–rabbit retina serum and guinea pig anti–guinea pig retina serum were prepared by footpad injection of homologous retina in a complete Freund’s adjuvant emulsion containing 10% (v/v) glycerol, pH 7.8. The combined suspensions were centrifuged at 50,000 × g for 30 min, concentrated to 16 ml, and applied in four separate 4 ml samples to a 95 by 2.6 cm Sephacryl S-200 column. The column was equilibrated in and eluted with T400N containing the above detergent buffer diluted 1:10 at a flow rate of 100 ml/hr into 4 ml fractions. Protein was monitored by dye-binding with Coomassie blue G-250, 14 and the equivalent fractions were pooled into 16 ml aliquots. The antigen-containing fractions were found by ELISA with GPaGP-ROS. The resulting preparation is similar in some respects to the particulate or "P" antigen reported by Wacker et al., 15~17 and we designate the antigen prepared in this manner as P to reflect this similarity. One immunologically active protein in the P antigen mixture is reported to be rhodopsin, 9 although the presence of other antigens has not been excluded.

**Preparation of antigens.** Forty bovine retinas were homogenized in T400N (5 mM Tris HCl and 400 mM NaCl, pH 7.8) and centrifuged at 50,000 × g for 1 hr. The supernatant was dialyzed against T400N, and saturated ammonium sulfate was added dropwise to a 60% final concentration. The suspension was spun at 50,000 × g for 30 min. The pellet was redissolved, dialyzed against T400N, and centrifuged at 400,000 × g for 1 hr. The resulting supernatant was applied to a calibrated 95 by 2.6 cm Sephacryl S-200 column equilibrated in T400N and eluted at a flow rate of 100 ml/hr. Four milliliter fractions were collected and monitored for absorbance at 280 nm. S antigen–positive fractions detected by counterimmunoelectrophoresis (CIE) using GPaGP-S were pooled, dialyzed against 50 mM Tris HCl, pH 7.5, concentrated to 5 ml, and applied to a 35 by 1 cm diethylaminoethyl (DEAE)-Sephacel column. The column was eluted with a 0.05M to 0.5M KCl gradient. The S antigen fractions detected by CIE using GPaGP-S were pooled, dialyzed against 50 mM Tris HCl, pH 7.5, and applied to a 35 by 1 cm phosphocellulose column that was eluted with a 0.05M to 0.5M KCl gradient. The S antigen fractions were found by ELISA with GPaGP-S, pooled, dialyzed, and concentrated.

The insoluble pellet from the 40 homogenized retinas was washed three times with T400N to remove residual soluble proteins and was rehomogenized three times in small volumes of 0.1M KCl, 0.05M Tris HCl, 0.5% NP-40, 0.2% deoxycholate (DOC), 5mM dithiothreitol (DTT), and 20% glycerol, pH 7.8. The combined suspensions were centrifuged at 50,000 × g for 30 min, concentrated to 16 ml, and applied in four separate 4 ml samples to a 95 by 2.6 cm Sephacryl S-200 column. The column was equilibrated in and eluted with T400N containing the above detergent buffer diluted 1:10 at a flow rate of 100 ml/hr into 4 ml fractions. Protein was monitored by dye-binding with Coomassie blue G-250, 14 and the equivalent fractions were pooled into 16 ml aliquots. The antigen-containing fractions were found by ELISA with GPaGP-ROS. The resulting preparation is similar in some respects to the particulate or "P" antigen reported by Wacker et al., 15~17 and we designate the antigen prepared in this manner as P to reflect this similarity. One immunologically active protein in the P antigen mixture is reported to be rhodopsin, 9 although the presence of other antigens has not been excluded.

**Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE).** Electrophoresis was done according to the method of Laemmli 18 except that 4% to 30% polyacrylamide gradients were used. The gels were stained with Coomassie blue R-250 and photographed on Kodak Technical Pan through a red filter. 

**ELISA.** ELISA was done according to the procedure of Voller et al. 19 with the modifications described below.

**Screening of column fractions by ELISA.** Ten microliters of each fraction were transferred into the wells of 96-well micro-ELISA plates (Flow Labs) followed by addition of 200 μl of coating buffer (15 mM Na₂CO₃, 35 mM NaHCO₃, and 0.02% NaN₃, pH 9.6). The plates were covered and incubated at 4° overnight. Phosphate-buffered saline–polysorbate (PBS-Tween) (140 mM NaCl, 1.5 mM KH₂PO₄, 8 mM Na₂HPO₄ · 12H₂O, 2.7 mM KCl, 0.05% Tween 20, and 0.02% NaN₃, pH
Fig. 1. Phosphocellulose elution profile (•••, dye-binding protein) of the S antigen-positive fractions from the DEAE-Sephacel column. The phosphocellulose column was eluted with a linear KCl gradient of 0.05M to 0.5M (-----). The fractions were assayed by ELISA and the average of two assays is presented (a-----a).

7.4) was used to rinse each well three times, and 200 μl of rabbit or guinea pig antiserum diluted as indicated in the text in PBS-Tween plus 1 mg/ml bovine serum albumin (PBS-Tween-BSA) were added. After incubation at 4° overnight, the plates were rinsed three times with PBS-Tween, and 200 μl of the appropriate anti-Ig·alkaline phosphatase conjugate (Miles) in PBS-Tween-BSA was added. The plates were incubated overnight at 4° and were rinsed twice with PBS-Tween and once with HEPES-saline (150 mM NaCl, 25 mM HEPES, pH 7.4), followed by addition of 200 μl of substrate (1M Tris base, 1M NaCl, 6 mM p-nitrophenyl phosphate, 2 mM MgCl₂, and 0.02% NaN₃, pH 9.5). The plates were incubated at 25° and read at 405 nm with the Titertek Multiskan (Flow Labs). ELISAs done on antigens in detergent buffers were modified to compensate for the inhibitory effects of detergents on the binding of antigens to the plates in coating buffer. This problem was overcome by including three changes of one half of the coating buffer-antigen mixture (i.e., 105 μl) at 6 hr minimum intervals. The rest of the procedure was unchanged.

Determination of human titers. Ten microliters of antigen solution containing 48 ng of S or 175 ng of P were dispensed into the wells of micro-ELISA plates. Doubling dilutions of the human sera were done in vertical columns from 1:50 to 1:6400. The human sera at a 1:25 dilution were preincubated with BSA at 1 mg/ml in PBS-Tween, which also contained calf serum at a dilution of 1:25 to absorb nonspecific anti-bovine activity commonly found in human sera. This absorption procedure removes all the anti-calf serum activity of the human sera. After overnight incubation at 4°, the wells were rinsed and 200 μl of goat anti-human IgG and IgM·alkaline phosphatase conjugate (GaHlgG·AP and GaHlgM·AP), each diluted at 1:1000 in PBS-Tween-BSA containing a 1:400 dilution of calf serum, were added.

Data analysis. Antibody titration curves were straightened by plotting on double-probability paper the eight points corresponding to the absorbance at each of the eight dilutions in the titrations and drawing the straight line that best fit the points. A dilution of 1:50 corresponds to a titer of 1, 1:100 corresponds to a titer of 2, 1:200 to a titer of 3, and so on. The titers were determined graphically as the dilution required to give an A₄₀⁵ nm over background of 0.3 (S antigen tests) or 0.5 (P antigen tests) as judged by the intersection of the straight line with the cutoff value in 1 hr of incubation at 25°. The higher cutoff used in the P antigen tests reflects the higher average titers found to this antigen(s). Titers of 0 or 9 were graphically obtained by extrapolation. The two-tailed Student's t test was used to determine whether the responses of any patient group differed from the normals. A paired-t analysis was also used to compare the prelaser and postlaser groups.

Results

Preparation of antigens for use in ELISA. The saline-soluble protein fraction of the bovine retinas prepared as described in Materials and methods was applied to a Sephacryl S-200 column, eluted, and assayed for antigenic activity by ELISA. Rabbit anti-rabbit
Fig. 2. ELISA of the phosphocellulose fractions from Fig. 1 for antigenic activity with GPaGP-S (—•—•) and NGPS (○—○), each at a dilution of 1:200. Goat anti-guinea pig IgG·alkaline phosphatase conjugate (1:1000) was used to develop the assays. Retina serum at a dilution of 1:100 was used to detect the presence of antigenic retinal proteins in these fractions. The assays were developed with a 1:200 dilution of goat anti-rabbit IgG·alkaline phosphatase conjugate (GaRlG·AP). The assays were read for $A_{405}$ nm, and the averaged results of two separate runs were used in conjunction with CIE using GPaGP-S to locate the S antigen. The fractions that were most positive by CIE and ELISA were taken for further purification on a DEAE-Sephacel column, and a sample was also taken for SDS-PAGE (Fig. 4, lane B). The DEAE-Sephacel fractions that were positive for S antigen by CIE using GPaGP-S were pooled for a subsequent run on a phosphocellulose column as shown in Fig. 1. The phosphocellulose fractions were assayed by ELISA with rabbit anti-rabbit retina serum at a dilution of 1:200 and GaRlG·AP at 1:400. The average of two assays is presented. Fig. 2 gives the results of an ELISA on the phosphocellulose column fractions, using GPaGP-S serum at a dilution of 1:200. Also shown are the results from normal guinea pig serum (NGPS) at the same dilution. It is interesting to note that the rabbit anti-rabbit retina serum detected an antigen in fractions 9 to 17 that was not found with the GPaGP-S serum (Fig. 2). The S antigen-positive fractions (38 to 48 from the phosphocellulose purification were pooled, dialyzed, and concentrated, and a sample was taken for SDS-PAGE. The detergent-soluble retinal extract was prepared as described above and was chromatographed on a detergent-equilibrated Sephacryl S-200 column as shown in Fig. 3, A. Fractions from the column were assayed for reactivity in the ELISA using GPaGP-ROS, rabbit anti-rabbit retina, guinea pig anti-guinea pig retina, NGPS, and normal rabbit sera (NRS). These results are presented in Fig. 3, B and C. The positive fractions (79 to 89) were pooled and concen-
A sample of the detergent-extracted antigen was taken for SDS-PAGE. Electrophoresis of the S antigen revealed a single major band with a molecular weight of approximately 50,000, whereas the P antigen was a heterogeneous preparation (Fig. 4). Rabbit, rat, bovine, monkey, human, and murine (unpublished observation) S antigens have been shown to cross-react with guinea pig S antigen using GPaGP-S in immunodiffusion. Although the S antigens from different species cross-react, they are not all equally immunopathogenic, indicating the presence of antigenic differences.20

Lack of cross-reactivity between the S and P antigen preparations. To eliminate the
possibility that the antigens were cross-
contaminated or cross-reactive, the ability of
an excess of S antigen to absorb the anti-P
binding activity of GPaGP-ROS serum and
vice versa was tested in the ELISA (Table I).
Only the homologous antigen was able to in-
hbit the binding between S antigen and
GPaGP-S and similarly the binding between
the P antigen preparation and GPaGP-ROS.
The significant anti-P response of even the
NGPS was interesting and is consistent with
the occasionally high titers of normal human
control sera to P antigen we have found.

Antibody titers of patients and controls.
Serum samples collected from patients diag-
nosed and categorized according to the guide-
lines above were assayed for anti-S and anti-P
activity in the ELISA. The results of the
uveitis patients and the normal controls are
presented in Table II and Fig. 5. Statistically
significant titers to S antigen were found in
the categories of iritis, toxoplasmosis, sar-
coidosis, and Behçet's syndrome, whereas
only the granulomatous and hypopyon sub-
categories of iritis, toxoplasmosis, sarcoidosis,
and VKH patients responded significantly to
the P antigen. In virtually all individual pa-
tients, regardless of clinical signs, elevated
levels of anti-S were accompanied by higher
levels of anti-P and vice versa. From the data
presented in Fig. 5 and Table II it can be
seen that some patients had very high titers
and others had no detectable responses. The
reason for this wide range of titers is unclear,
but it was not a result of variability in the
 technique, since the reliability of the assay
system was verified by repeated blood sam-
ples and assays in which repeat samples from
the same patient had a much lower variabil-
ity. Because the average titers were low, we
have made use of several types of controls in
an effort to lend credibility to these results.
An obvious choice was to test a group of five
patients who had nonuveal ocular inflamma-
tions, including episcleritis, conjunctivitis,
and corneal laceration. This group had titers
indistinguishable from those of the normal
controls (Table III). A second control group
consisted of uveitis patients being treated
with corticosteroids, either systemic or retro-

Fig. 4. SDS-PAGE of the antigens and molecular
weight markers. Lanes A and F, Molecular weight
markers, including phosphorylase B, BSA, oval-
bumin, carboxy anhydrase, soybean trypsin in-
hibitor, and a-lactalbumin; lane B, sample of
Sepharcll S-200 pool of fractions 55 to 61; lane C,
unrelated; lane D, S antigen; lane E, P antigen.
bulbar. The titers of these patients were de-
pressed significantly in their anti-S response.
Most of these patients had elevated anti-S and
anti-P levels prior to steroid therapy.

A final control group was included to exa-
mire the possibility that other types of retinal
cellular disruption might induce autoimmune
responses to retinal antigens. This group
consisted of patients receiving laser photocoagulation treatment for diabetic retinop-
athy. Blood samples were obtained both be-
fore and after laser treatment, and antibody
titers were determined (Table IV and Fig. 5).
The titers of the postlaser blood samples
were elevated over those of normal controls
in their anti-S activity (p < 0.002), and their
anti-S response was elevated (p < 0.05) rela-
tive to the prelaser controls as determined by
the paired-t analysis, indicating that the laser
treatment increased the patients' titers.

We have searched for "common denomina-
tors" in the patient population in hopes of
finding a correlation between antibody titers
and some characteristic of the patients. Age,
sex, HLA type (20 of these patients have been
typed), degree of inflammation, and the
chronicity of the inflammation did not corre-
Fig. 5. Antibody titers of uveitis patients and controls to the S and P antigens. A, Pooled toxoplasmosis; B, anterior and posterior toxoplasmosis; C, posterior toxoplasmosis only; D, pooled sarcoidosis; E, herpes keratouveitis; F, pars planitis; G, normal controls; H, pooled iritis, I, nongranulomatous iritis; J, granulomatous iritis; K, hypopyon iritis; L, prelaser control values; M, postlaser values; N, normal controls. Horizontal dashes indicate the means of each group. The means, standard deviations, t probability levels, and other data for these groups are shown in Table II.

Table I. Lack of cross-reactivity between the S and P antigen preparations

| Guinea pig serum | Antigen present in preincubation | Test antigen in ELISA | A\textsubscript{405 nm} (mean ± S.D.)
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>GP\textsubscript{G}GP-S</td>
<td>None</td>
<td>S</td>
<td>1.290 ± 0.058</td>
</tr>
<tr>
<td></td>
<td>S\textsuperscript{a}</td>
<td>S</td>
<td>0.582 ± 0.048</td>
</tr>
<tr>
<td></td>
<td>P\textsuperscript{b}</td>
<td>S</td>
<td>0.328 ± 0.046</td>
</tr>
<tr>
<td></td>
<td>None</td>
<td>S</td>
<td>0.221 ± 0.018</td>
</tr>
<tr>
<td></td>
<td>None</td>
<td>P</td>
<td>0.320 ± 0.021</td>
</tr>
<tr>
<td>GP\textsubscript{G}GP-ROS</td>
<td>None</td>
<td>S</td>
<td>0.087 ± 0.025</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>S</td>
<td>0.086 ± 0.015</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>S</td>
<td>0.088 ± 0.014</td>
</tr>
<tr>
<td></td>
<td>None</td>
<td>P</td>
<td>1.290 ± 0.058</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>P</td>
<td>1.240 ± 0.041</td>
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<tr>
<td></td>
<td>None</td>
<td>P</td>
<td>0.562 ± 0.048</td>
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<tr>
<td></td>
<td>None</td>
<td>S</td>
<td>0.087 ± 0.022</td>
</tr>
<tr>
<td>NGPS</td>
<td>None</td>
<td>S</td>
<td>0.328 ± 0.046</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Sera used at 1:500 dilution.
\textsuperscript{b}Developed with a sandwich using rabbit anti-guinea pig Ig at 1:500 and CoRlgG-AP at 1:2000.
\textsuperscript{c}Average of 6 assays.
\textsuperscript{d}48 ng/well of S or 175 ng/well of P.
\textsuperscript{e}1.5 \mu g/ml of S or 6 \mu g/ml of P.
Table II. Titers of uveitis patients to bovine retinal S and P antigens

<table>
<thead>
<tr>
<th>Diagnostic category</th>
<th>Anti-S antigen</th>
<th>Anti-P antigen</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± S.D.</td>
<td>E.V.</td>
</tr>
<tr>
<td>Herpes keratouveitis</td>
<td>2.13 ± 1.23</td>
<td>4.2, 0.6</td>
</tr>
<tr>
<td>Pars planitis</td>
<td>2.24 ± 1.32</td>
<td>4.6, 0.2</td>
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<tr>
<td>Iritis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All types</td>
<td>2.47 ± 1.22</td>
<td>5.4, 0.2</td>
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<tr>
<td>Nongranulomatous</td>
<td>2.47 ± 1.32</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Granulomatous</td>
<td>2.63 ± 0.77</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hypopyon</td>
<td>2.90 ± 0.90</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fuchs’ heterochromia</td>
<td>1.38 ± 0.81</td>
<td>NS</td>
</tr>
<tr>
<td>Toxoplasmosis</td>
<td></td>
<td></td>
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<tr>
<td>Pooled data</td>
<td>2.46 ± 0.71</td>
<td>3.9, 1.1</td>
</tr>
<tr>
<td>Anterior and posterior</td>
<td>2.72 ± 0.65</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Posterior only</td>
<td>2.02 ± 0.67</td>
<td>NS</td>
</tr>
<tr>
<td>Sarcoidosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pooled data</td>
<td>3.38 ± 1.04</td>
<td>4.8, 1.9</td>
</tr>
<tr>
<td>Anterior and posterior</td>
<td>3.39 ± 1.13</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Posterior only</td>
<td>3.30 NA</td>
<td>NA</td>
</tr>
<tr>
<td>Behcet’s syndrome</td>
<td>2.53 ± 0.39</td>
<td>3.1, 2.2</td>
</tr>
<tr>
<td>VKH</td>
<td>1.87 ± 0.58</td>
<td>2.3, 0.6</td>
</tr>
<tr>
<td>Normals</td>
<td>1.55 ± 0.49</td>
<td>2.0, 0.2</td>
</tr>
</tbody>
</table>

E.V. = extreme values; NS = not significant; NA = not applicable.

See Table II for explanations.

Table III. Anti-S and anti-P titers of control patients

<table>
<thead>
<tr>
<th>Diagnostic category</th>
<th>Anti-S antigen</th>
<th>Anti-P antigen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± S.D.</td>
<td>E.V.</td>
</tr>
<tr>
<td>Nonuveal ocular inflammations</td>
<td>1.42 ± 0.76</td>
<td>2.1, 0.2</td>
</tr>
<tr>
<td>Uveitis patients treated with steroids</td>
<td>0.92 ± 0.78</td>
<td>2.2, 0</td>
</tr>
<tr>
<td>Normal</td>
<td>1.55 ± 0.49</td>
<td>2.0, 0.2</td>
</tr>
</tbody>
</table>

See Table II for explanations.

Discussion

The data presented in this report demonstrate that the serum antibody titers of several different types of uveitis patients are elevated against bovine S antigen and against a detergent extract of bovine retina similar to the P antigen reported by Wacker et al.15-17 Although the average titers were numerically elevated in all the types of uveitis we investigated, only some of the elevations were statistically significant. It is possible that if the sizes of the uveitis patient groups and the control group had been larger, all the elevations would have been statistically significant. As we expand the definitive uveitis patient population, i.e., data base, we hope to be able to answer this question. Another factor influencing the statistical evaluations was the...
patient-to-patient variation in titer. Only the normal controls vs. S antigen gave tightly clustered results, whereas the anti-P responses in all categories showed wide variability. In many respects these results are similar to those we have found in animal models of EAU. The anti-P responses of guinea pigs and rabbits also tended to be higher than the anti-S responses. Experiments are in progress to determine whether there are antigens in the P preparation other than rhodopsin being recognized by the uveitis sera. We have also found that unimmunized control animals exhibit similar variability and often had surprisingly high anti-P titers (Table I and unpublished observations). The reason for this observation is uncertain but could be related to the normal turnover of outer segments and the potential release of antigenic material in this process. Low levels of specific antibodies to self components have previously been observed and may be related to physiologically normal debris-clearing activities. These observations underscore the necessity to view “autoimmune responses” critically before attributing pathologic effects or autoimmune disease to the mere presence of an autoimmune response. Even the ability to induce an experimental autoimmune disease model after immunization with a specific antigen does not prove that the same causal relationship exists between that antigen and the human disease. Since the presumed causative agents of the types of uveitis represented in this study are very different (toxoplasmosis, herpes keratouveitis, or unknown), it is possible that the elevated S and P titers observed in different categories reflect an epiphenomenon and are unrelated to the mechanism of the disease process. Although both the S and P antigens are uveitogenic in experimental allergic uveitis, they may not be etiologic in the case of human uveitis.

The diabetic group consisted of six individuals, all of whom were assayed before their first laser treatment and after one, and in some cases two, laser treatments. Their postlaser S antigen titers were significantly elevated over normal controls, whereas the P antigen titers were not significantly elevated at the 0.05 level. The group of prelaser and postlaser treatment diabetic patients was included in this study to investigate the role of “nonuveitis” type retinal trauma in the induction of autoimmune responses to retinal antigens. Our results suggest that autoimmune responses to retinal antigens may be a result of their release, either by laser photocoagulation or by other means. It is also possible that the retinal changes of early diabetic retinopathy may in themselves produce enough cellular disruption to release retinal antigen and initiate a low-grade autoimmune response. The anti-S titers were somewhat higher in the prelaser group as compared to normals, but the differences were not statistically significant. Because of the potential importance of the observation in the diabetic group, a separate study with larger numbers of well-characterized diabetic patients and controls is now underway and will be reported separately. A similar type of autoimmune response may be operative in certain forms of uveitis in response to tissue disruption secondary to inflammation. Similar concern was expressed by Brinkman et al., who observed anti-retinal responses in retinitis

Table IV. Anti-S and anti-P titers of laser-photocoagulated patients

<table>
<thead>
<tr>
<th>Diagnostic category</th>
<th>Anti-S antigen</th>
<th>Anti-P antigen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± S.D.</td>
<td>E.V.</td>
</tr>
<tr>
<td>Prelaser</td>
<td>1.98 ± 0.88</td>
<td>3.5, 1.2</td>
</tr>
<tr>
<td>Postlaser</td>
<td>2.79 ± 1.03</td>
<td>4.3, 0.9</td>
</tr>
<tr>
<td>Normal</td>
<td>1.55 ± 0.49</td>
<td>2.0, 0.2</td>
</tr>
</tbody>
</table>

*Results of paired-t analysis of prelaser and postlaser patients. See Table II for additional explanations.
pigmentosa. The possibility that anti-retinal immune responses are frequently secondary or epiphenomena with respect to the mechanisms underlying uveitis is supported by experiments in which reactivity was found in patients with penetrating eye injuries,\textsuperscript{25} retinal detachment,\textsuperscript{26} and pigmentary retinal degenerations.\textsuperscript{27}

We have also observed that there was an apparent lack of a direct correlation between the activity levels of uveitis patients and their titers to the S and P antigens. We are working to explore this aspect in a longitudinal study that will examine patients over a prolonged period of time during which several serial serum samples can be obtained and correlated with the clinical course. On the basis of a limited number of these patients, we have some preliminary evidence that the antibody titer rises 2 or more weeks after the patient's activity level has peaked. We hope to be able to confirm this impression with a larger sample of patients.

Previous studies of human uveitis and EAU together with this report demonstrate that autoreactive cell-mediated responses\textsuperscript{28–30} and autoantibodies\textsuperscript{11,12} are present in uveitis and that complement\textsuperscript{31} plays some role in EAU. Considerably more work will be required to establish which of the many effector mechanisms of the immune response is responsible for the immunopathogenic processes observed in uveitis and to determine whether the anti-S and anti-P antibody responses actually contribute to the immunopathogenesis of human uveitis.

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REFERENCES


