Adoptive Transfer of Experimental Autoimmune Uveoretinitis in Rats

Immunopathogenic Mechanisms and Histologic Features

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In order to learn about the immunopathogenic mechanisms of experimental autoimmune uveoretinitis (EAU), the capacity of lymphocytes to transfer the disease was studied. EAU was transferred to naive syngeneic rats by intraperitoneal injection of spleen or lymph node (LN) cells from S-antigen immunized rats, following their incubation in culture with either S-antigen or concanavalin A (Con A). In contrast, the same cells did not cause inflammatory changes in the recipient eyes when injected intravitreally. The identity of the lymphocytes that transfer EAU was determined by using monoclonal antibody enriched subsets of lymphocytes. EAU was transferred by the subset of helper/inducer T-cells, but not by T-cells of the suppressor/cytotoxic subset. Recipient rats of spleen or LN cells cultured with S-antigen exhibited both humoral and cellular immune responses to S-antigen. On the other hand, recipients of spleen cells cultured with Con A developed only cellular immune response to S-antigen. Yet, both groups of recipients fully developed EAU. The clinical and histologic changes in recipient rats closely resembled those in rats in which EAU was induced by active immunization. Severe tissue damage occurred at the photoreceptor cell layer, but inflammatory infiltration also was found in other ocular tissues. Involvement of polymorphonuclear leukocytes (PMNs) was noted throughout ocular tissues even in the eyes of recipients with no detectable antibodies to S-antigen, suggesting that the ocular PMNs infiltration in rats is not necessarily the result of an Arthus-like inflammatory process. Invest Ophthalmol Vis Sci 26:1-9, 1985

Experimental autoimmune uveoretinitis (EAU) is an ocular inflammatory disease that can be induced in a variety of experimental animals by immunization with the retinal specific S-antigen. The disease is of interest as a model for certain types of inflammatory eye conditions in humans. Little is known about the immunopathogenic mechanisms of EAU. Previous studies have indicated that T-lymphocytes play the major role in the pathogenesis of EAU: (1) athymic nude rats did not develop the disease following immunization with S-antigen and (2) development of EAU was inhibited completely by treatment with cyclosporine, an immunosuppressive agent selective for T-lymphocyte functions. On the other hand, data reported by Marak and co-workers suggested that complement-fixing antibodies play a pathogenic role as well, since treatment with cobra venom factor could modulate the histopathologic changes in animals, which develop EAU.

Useful information concerning the immunopathogenic mechanisms of autoimmune diseases has been obtained in studies in which the diseases are transferred adoptively by serum or lymphoid cells from immunized donors. Although chorioretinal changes were induced in naive guinea pigs injected intravitreally or subconjunctivally with hyperimmune sera, no disease could be transferred by systemic injection of such sera (and our unpublished data). More reproducible have been attempts at transferring EAU by lymphoid cells. Aronson and McMaster and Meyers successfully transferred certain types of EAU from immunized guinea pigs to naive recipients, using large numbers of freshly harvested lymphoid cells. The efficacy of disease transfer by lymphoid cells...
cells recently has been found to be increased strikingly by preculturing the cells with the organ-specific antigen or a lectin such as concanavalin A (Con A). Most studies on this topic have been carried out with animals affected by experimental allergic encephalomyelitis (EAE), but preliminary data have been reported showing that this procedure is similarly successful in transferring EAU in rats and guinea pigs. This paper describes in detail our extended study concerning the immunopathogenic mechanisms and histologic features of adoptively transferred EAU in Lewis rats.

Materials and Methods

Animals

Lewis rats were purchased from M. A. Bioproducts (Walkersville, MD). Male or female rats between 9 and 14 weeks of age were used. All procedures used in this study conform to the ARVO Resolution on the Use of Animals in Research.

Immunization of Donor Rats

S-antigen was prepared using bovine retinas according to the method of Dorey et al. The antigen was emulsified (1:1) in complete Freund's adjuvant (CFA), containing Mycobacterium tuberculosis H37Ra at a concentration of 2.0 mg/ml. As a control for S-antigen, bovine gamma globulin (BGG) (Miles Laboratories; Elkhart, IN), or Dulbecco's phosphate buffered saline (PBS) were used in some experiments. A total volume of 0.2 ml/rat, containing 50 μg of S-antigen or 100 μg of BGG, was injected into four footpads of rats. The animals were killed between 12 and 14 days after immunization and the spleen and the draining LN were collected.

In Vitro Culture of Lymph Node (LN) and Spleen Cells

The spleen and LN from donor rats were teased gently in RPMI-1640 medium with HEPES (GIBCO, Grand Island, NY) to obtain cell suspensions. The cells were washed twice and suspended to a concentration of 2 X 10^6 cells/ml in the RPMI-1640 medium, supplemented with streptomycin (100 μg/ml), penicillin (100 U/ml), 5% heat-inactivated fetal calf serum (FCS, GIBCO), and 2-mercapto-ethanol (5 x 10^-5 M). Cultures were set up in 12-well cluster plates (Costar, Cambridge, MA; or Linbro, Hamden, CT) and consisted of 2-ml cell suspension, with or without stimuli. The stimulants included S-antigen (2 μg/ml), Con A (1 μg/ml, Miles Yeda; Rehovot, Israel) or BGG (10 μg/ml). Following incubation at 37°C with 100% humidity and 5% CO₂ in air for 3 days, the cells were harvested, washed twice, and injected to naive syngeneic rats of the same sex of the donors intraperitoneally. In some experiments, the cells were injected into the vitreous using a 30G needle under an operation microscope.

Fractionation of Cultured Cells

Subsets of helper/inducer or suppressor/cytotoxic T-lymphocytes were enriched using monoclonal antibodies according to the method described by Mage et al. Briefly, the cell suspensions following 3 days incubation were harvested gently, washed twice, and resuspended to a concentration of 2 x 10^7 cells/ml in RPMI-1640 medium with 5% FCS. The cell suspensions were incubated in a 100-mm tissue culture dish (1 x 10^8 cells/dish), which had been coated with rabbit anti-mouse immunoglobulin (100 μg/ml; Dakopatts, Copenhagen, Denmark). Following incubation at 4°C for 70 min, nonadherent cells (Ig-negative cells) were collected carefully, washed twice, and suspended to a concentration of 1 x 10^7 cells/ml in RPMI-1640 medium with 5% FCS. Aliquots of these cell suspensions then were incubated with mouse IgG monoclonal antibody against rat nonhelper T-cells, clone OX8 (50 μg/ml, Accurate Chemical & Scientific Co.; Westbury, NY), or mouse IgG₁ monoclonal antibody against rat-helper T-cells, clone W3/25 (50 μg/ml, Accurate Chemical & Scientific Co.). Following incubation (4°C, 45 min), the cell suspensions were washed twice to remove excess of antibodies and suspended to a concentration of 1 x 10^7 cells/ml in RPMI-1640 medium with 5% FCS. The cell suspensions (3 ml/dish) were incubated in the 100-mm tissue culture dishes coated with rabbit anti-mouse immunoglobulins. Following incubation at 4°C for 70 min, nonadherent cells were harvested carefully, washed twice, suspended in RPMI-1640 medium with 1% FCS, and injected intraperitoneally to naive syngeneic rats. Examination of the fractionated cell suspensions demonstrated a high level of specific staining with either the OX8 or W3/25 antibodies (suppressor/cytotoxic or helper/inducer subsets, respectively).

Evaluation of Immune Responses in Recipient Rats

The recipient rats were skin tested between days 6 and 9 after cell injection by an intracutaneous injection of S-antigen, 100 μg in 0.1 ml PBS. Skin responses were read after 3 hr (Arthus) or 24 hr (delayed type hypersensitivity: DTH). The responses were assessed according to the size of induration (diameter, in mm). Blood samples, collected before
skin testing, were used to measure the level of antibodies to S-antigen, using the enzyme-linked immunosorbent assay (ELISA). Briefly, 96-well polystyrene plates (Dynatech Laboratories, Inc.; Alexandria, VA) were coated with S-antigen (50 ng per well in PBS). After 1 hr incubation at 37°C with the antigen, and washing five times with PBS containing 0.05% Tween 20 (PBS-Tween), the wells were incubated with PBS-Tween at room temperature for another hour. The wells were washed again as described above and serum samples, 100 μl, diluted with PBS-Tween were added. Following incubation (1 hr, 37°C), the wells were washed five times with PBS-Tween. One hundred microliters of a 1:1000 dilution of peroxidase conjugated anti-rat IgG (Miles-Yeda Research Products, Israel) was added to each well, and the plates were incubated at room temperature for 1 hr. Excess conjugate was washed out and 100 μl of O-phenylenediamine (Sigma Chemical Co.; St. Louis, MO) (0.4 mg/ml in citrate PO4 buffer containing 3% H2O2) was added to the wells. Fifteen minutes after adding the substrate, the reaction product was measured by absorbance at 410 nm using an automated device (Titerek Multiskan MC, EFLAB; Helsinki, Finland). Antibody levels were expressed as the absorbance values at 1:640 dilution of each serum sample.

Evaluation of EAU

Clinical signs of EAU were monitored daily after cell transfer, with and without a slit-lamp microscope. Eyes with EAU were enucleated at various intervals after the onset of the disease. Recipient rats that did not develop uveitis clinically were followed up until day 15 postinjection and killed thereafter. Eyes were prepared for histologic examination as described previously.

Results

Adoptive Transfer of EAU by Intraperitoneal Injection of Sensitized Cells

Table 1 summarizes data of repeated experiments testing the induction of EAU in recipient rats by intraperitoneal injection of various types of sensitized lymphoid cells. The onset of EAU was determined by the appearance of clinical changes and the disease

<table>
<thead>
<tr>
<th>Donor sensitization</th>
<th>Source organ</th>
<th>Stimulus in culture</th>
<th>Number of cells (×10^3)</th>
<th>Rats with EAU</th>
<th>Day of onset</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-antigen/CFA</td>
<td>Lymph nodes</td>
<td>S-antigen</td>
<td>5-9</td>
<td>15/17</td>
<td>6.7 ± 0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Concanavalin A</td>
<td>10-15</td>
<td>6/11</td>
<td>7.2 ± 0.7</td>
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<tr>
<td></td>
<td></td>
<td>None</td>
<td>8</td>
<td>0/4</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Uncultured</td>
<td>10</td>
<td>0/3</td>
<td>—</td>
</tr>
<tr>
<td>Spleen</td>
<td>S-antigen</td>
<td>5-9</td>
<td></td>
<td>8</td>
<td>6.8 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>Concanavalin A</td>
<td>5-9</td>
<td></td>
<td>50-100</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>None</td>
<td>8</td>
<td></td>
<td>0/3</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Uncultured</td>
<td>0/4</td>
<td></td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0/3</td>
<td></td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>PBS/CFA</td>
<td>Lymph nodes</td>
<td>S-antigen</td>
<td>8</td>
<td>5/5</td>
<td>6.6 ± 0.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Concanavalin A</td>
<td>2</td>
<td>0/2</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Spleen</td>
<td>Concanavalin A</td>
<td>2</td>
<td>0/2</td>
<td>—</td>
</tr>
</tbody>
</table>

* The activity of helper/inducer cells was determined in three repeated experiments and that of suppressor/cytotoxic lymphocytes in two experiments.

In one experiment, both lymphocyte subsets from the same donors were tested.

Table 2. Adoptive transfer of EAU by intraperitoneal injection of purified subsets of T-cells

<table>
<thead>
<tr>
<th>Source organ</th>
<th>Stimulus in culture</th>
<th>Subset of T-cells</th>
<th>Number of cells (×10^3)</th>
<th>Rats with EAU</th>
<th>Day of onset</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymph nodes</td>
<td>S-antigen</td>
<td>Helper/inducer</td>
<td>2-3</td>
<td>5/5</td>
<td>6.6 ± 0.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Suppressor/cytotoxic</td>
<td>6</td>
<td>0/2</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td>—</td>
</tr>
<tr>
<td>Spleen</td>
<td>Concanavalin A</td>
<td>Helper/inducer</td>
<td>3-4</td>
<td>4/4</td>
<td>5.6 ± 0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Suppressor/cytotoxic</td>
<td>6</td>
<td>0/2</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td>—</td>
</tr>
</tbody>
</table>

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occurrence was verified by histologic examination. The ocular changes in recipient rats are described in detail below. LN cells cultured with S-antigen were the most potent to transfer EAU; a great majority of recipients developed EAU when injected with 5–9 × 10⁷ of these cells and about one-half developed disease following injection with 2–4 × 10⁷. On the other hand, LN cells cultured with Con A did not transfer EAU even at 10–15 × 10⁷ per recipient. Spleen cells from S-antigen immunized donors transferred EAU following culturing with either S-antigen or Con A, but with lower efficacy than that of the LN cells; approximately one-third only of recipients of 5–9 × 10⁷ of these cells developed EAU. Stimulation in vitro of the lymphoid cells was found mandatory, since cells from S-antigen-immunized donors did not transfer EAU when injected without having been cultured or after incubation without S-antigen or Con A. Similarly ineffective were LN or spleen cells from donors immunized with CFA and cultured with S-antigen or Con A, respectively.

The identity of lymphocytes responsible for transferring EAU was determined by using subsets of lymphocytes, fractionated from in vitro stimulated LN or spleen cells (Table 2). Only the enriched subset of helper/inducer T-cells from either LN or spleen cells was found effective in transferring EAU, while similar numbers of cells of the suppressor/cytotoxic subset had no effect.

Clinical Features of EAU in Recipient Rats

A high level of conformity was found among affected recipients of lymphoid cells of different types in their clinical features. Moreover, a remarkable similarity was noted between the clinical features in the recipients and those in rats actively immunized

Fig. 1. Early histologic changes in the retina of a recipient of LN cells (8 × 10⁷) cultured with S-antigen. The eye was enucleated on day 6 after cell transfer, 1 day after disease onset (hematoxylin and eosin, ×120).

Fig. 2. Histologic changes in the anterior segment of the eye shown in Figure 1. A, Heavy cell infiltration was found in the posterior chamber, the iris and the ciliary body, whereas the anterior chamber was minimally infiltrated (hematoxylin and eosin, ×40). B, the cells infiltrating the posterior chamber were mostly PMNs (hematoxylin and eosin, ×400).
with S-antigen. Indeed, the only noticeable differences between the two groups of rats were (1) the onset of disease was always much earlier in recipients (5–8 days) than in actively immunized rats (11–15 days), and (2) an unilateral involvement was found in approximately ¼ of the recipients, while bilateral involvement was observed in virtually all actively immunized rats. The first clinical symptoms were usually hyperemia of the conjunctiva, pericorneal and iris vasodilation, and appearance of exudate and cells in the anterior chamber and the vitreous. Within 2–4 days after the onset, the ocular inflammation reached its peak with clinical signs such as protruding eyes due to severe periorcular inflammation, corneal edema, intense cell infiltration in the anterior chamber as well as in the vitreous, and posterior synechia of the iris. In many cases, cells in the anterior chamber formed hypopyon iridis. In about 2 weeks after onset of disease, inflammatory activity gradually subsided, cells in the anterior chamber disappeared, and the inflamed eye became quiet clinically.

**Histopathologic Features of EAU in Recipient Rats**

Histopathologic changes in the eyes of recipient rats were generally similar to those of rats actively immunized with S-antigen. Early histologic changes in the retina of recipients of LN cells cultured with S-antigen were exudate formation and mild accumulation of inflammatory cells in the photoreceptor cell layer (Fig. 1). The retina became detached with accumulation of serum underneath. Infiltrating cells in the damaged photoreceptor cell layer and in the subretinal fluid were a mixture of polymorphonuclear leukocytes (PMNs) and histiocytes. The choroidal tissue was minimally affected by inflammation in the early stage of the disease. Early changes in the anterior compartment of these rats were characterized by a heavy infiltration of inflammatory cells in the iris, ciliary body and posterior chamber (Fig. 2). Infiltrating cells were mainly PMNs and few mononuclear cells.

Figure 3 shows a typical example of histologic changes in the posterior portion of an eye manifesting maximal clinical uveitic changes, 3 days after disease onset. Photoreceptor cells were mostly degenerated and this layer and perivasculary regions of the retina were infiltrated intensely by inflammatory cells. The retina was detached totally from the choroid. Cells found in the retina and subretinal space were a mixture of PMNs and histiocytes, but the latter were more predominant. The vitreous and the choroid also were infiltrated with similar cells. At later stages, 2 or 3 weeks following the onset of the disease, no inflammatory cells were observed in the eye, though the photoreceptor cells were completely absent (not shown).

**Fig. 3.** Histologic changes in the posterior portion of a recipient's eye manifesting maximal clinical changes. The rats received $6 \times 10^7$ LN cells cultured with S-antigen and developed EAU 7 days later. The eye was enucleated 3 days after the disease onset (hematoxylin and eosin, x150).

Histopathologic features of eyes of animals that received spleen cells cultured with Con A or purified subset of helper/inducer T-lymphocytes were fundamentally similar to those described above. However, the proportion of various types of inflammatory cells found in this group of recipient rats were somewhat different from those found in recipients of LN cells cultured with S-antigen: rats of the former group showed more intense involvement of histiocytes in the retina as well as in the anterior uvea than found in rats of the latter group. Figures 4 and 5 show an EAU eye of a recipient of the helper/inducer T-lymphocytes purified from spleen cells cultured with Con A. The eye was enucleated on the day of disease onset, 5 days after cell transfer. Unlike the heavy
Fig. 4. Early changes in the anterior segment of an eye of a recipient of the helper/inducer T-lymphocytes ($4 \times 10^7$) purified from spleen cells cultured with Con A. A, Severe cell infiltration was found in the posterior chamber, the iris, and the ciliary body. Very shallow anterior chamber in the picture was due to collapse during fixation (hematoxylin and eosin, $\times 35$). B, Cells found in the posterior chamber, consisting of histiocytes (H) and PMNs (P) (hematoxylin and eosin, $\times 350$).

Fig. 5. Histologic changes in the posterior portion of the same eye of Figure 4. The photoreceptor cell layer and the subretinal space were infiltrated by a mixture of histiocytes and PMNs. Number of mononuclear cells was much more than that of PMNs throughout the retina (hematoxylin and eosin, $\times 130$).

involvement of PMNs in the anterior uvea of recipients of LN cells cultured with S-antigen (Fig. 2), many histiocytes and lymphocytes, in addition to PMNs, were found in the anterior uvea of this eye (Fig. 4). The retina also exhibited similar trend of cell involvement (Fig. 5). The aforementioned differences among actively immunized rats and recipients of various lymphoid cell populations in their pattern of involvement of PMNs and mononuclear leukocytes are summarized in Table 3.

**Immune Responses in Recipient Rats**

Table 4 summarizes the immune responses to S-antigen in recipient rats that developed EAU. All rats exhibited positive DTH responses to S-antigen. On the other hand, positive Arthus responses and serum antibodies to S-antigen were found only in recipient rats receiving lymphoid cells cultured with S-antigen, while no antibody activity could be detected in recipients of cells cultured with Con A. Recipients of enriched subsets of helper/inducer lymphocytes exhibited immune response patterns similar to those found in recipients of the respective unfractionated lymphocyte population.
Table 3. Inflammatory cells in intraocular lesions

<table>
<thead>
<tr>
<th>Rat treatment</th>
<th>Number of eyes examined</th>
<th>Anterior uvea</th>
<th>Retina</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>PMNs</td>
<td>Histiocytes</td>
</tr>
<tr>
<td>Active immunization with S-antigen/CFA</td>
<td>18</td>
<td>++++*</td>
<td>+</td>
</tr>
<tr>
<td>Transfer of LN-S antigen†</td>
<td>16</td>
<td>++++</td>
<td>+</td>
</tr>
<tr>
<td>Spleen-S-antigen</td>
<td>7</td>
<td>++++</td>
<td>+</td>
</tr>
<tr>
<td>Spleen-Con A</td>
<td>10</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>LN-S-antigen (helper/inducer)</td>
<td>10</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Spleen-Con A (helper/inducer)</td>
<td>8</td>
<td>++</td>
<td>+++</td>
</tr>
</tbody>
</table>

* Intensity of inflammatory cells was graded by a scale of 0 to +++.† LN-S-antigen; lymph node cells cultured with S-antigen. Spleen—S antigen; spleen cells cultured with S-antigen. Spleen—Con A; spleen cells cultured with concanavalin A.

Intravitreal Injection of Sensitized Cells

LN cells cultured with S-antigen that transferred EAU when injected intraperitoneally were injected into the vitreous to examine whether the cells had a direct pathogenic activity. As a control, LN cells from rats immunized with BGG and cultured with this antigen were used. Twenty microliters of cell suspensions, containing 2–3 x 10⁶, were injected into the vitreous of one eye, while the other eye did not receive injection. None of the nine rats receiving LN cells cultured with S-antigen or the six rats injected with LN cells cultured with BGG developed any typical signs of EAU clinically or histologically in either the injected or uninjected eyes. Figure 6A shows an eye that received intravitreal injection of LN cells cultured with S-antigen and was enucleated 4 days after the injection. Many cells, mainly lymphocytes, were observed in the vitreous and the retina, especially around the retinal vein. However, no inflammatory changes were found in the photoreceptor cell layer. The cells found in the eye are presumably not inflammatory cells but rather the injected cells. This assumption is based on the following findings. First, cells in the lesions were lymphocytes, whereas cells in EAU eyes were mainly PMNs and histiocytes as described above. Second, numbers of these lymphocytes decreased as time passed. An example of an eye on day 8 postintravitreal injection is shown in Figure 6B where cells in the eye were much fewer than those on day 4 postinjection (Figure 6A). Third, a very similar cell accumulation was found in control eyes that received intravitreal injection of LN cells cultured with BGG (Figure 6C).

Discussion

The present study demonstrates that LN or spleen cells from rats immunized with S-antigen in CFA can transfer EAU to naive syngeneic rats following their incubation with S-antigen (LN and spleen cells) or Con A (spleen cells). The results also show that both in vivo sensitization with S-antigen and in vitro stimulation are required for successful transfer of EAU. Requirement for in vitro stimulation also was reported in adoptive transfer of EAU in the guinea pig, as well as in other autoimmune diseases, such as EAE and experimental autoimmune thyroiditis (EAT). The mechanism by which in vitro culture with specific antigen or a T-cell mitogen (Con A) generates effector cells has not been clarified yet. It is noteworthy that the cells sensitized in vivo with S-antigen and activated in vitro with the antigen were uveitogenic only when injected systemically, while no EAU was induced by these cells when injected into the vitreous. This finding is unclear and could be attributed to at least two different mechanisms: (1) the lymphoid cells injected into the vitreous have no

Table 4. Immune responses in recipient rats*

<table>
<thead>
<tr>
<th>Source organ</th>
<th>Stimulus in culture</th>
<th>Population of cells</th>
<th>Skin responses to S-antigen (mm diameter)</th>
<th>Antibodies to S-antigen†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymph nodes</td>
<td>S-antigen</td>
<td>Total</td>
<td>10.6 ± 1.2 (N = 20)</td>
<td>0.375 ± 0.109 (N = 5)</td>
</tr>
<tr>
<td>Spleen</td>
<td>S-antigen</td>
<td>Total</td>
<td>4.3 ± 1.5(6)</td>
<td>0.272 ± 0.03(3)</td>
</tr>
<tr>
<td></td>
<td>Concanavalin A</td>
<td>Total</td>
<td>0(6)</td>
<td>&lt;0.05(4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Helper/inducer T-cells</td>
<td>0(4)</td>
<td>&lt;0.05(4)</td>
</tr>
</tbody>
</table>

* All rats included in the Table developed EAU.
† Serum antibody level was measured by ELISA (see text). Mean level of serum antibodies to S-antigen in rats actively immunized with S-antigen was 1.02.
Fig. 6. Histologic changes of eyes injected into the vitreous with sensitized cells. A and B, eyes injected with LN cells sensitized and cultured with S-antigen, 4 and 8 days after injection, respectively. C, an eye 4 days after being injected with LN cells sensitized and cultured with BGG (hematoxylin and eosin, ×160).

access to the retinal depot of S-antigen, or (2) sensitized lymphoid cells do not infiltrate the affected ocular tissues immediately upon their systemic injection but, rather, may require an additional initiation or recruitment process in another location, perhaps a lymphoid organ. These hypotheses are currently under investigation.

Data recorded here provide new evidence to support the notion that T-cell-mediated processes play the major role in the pathogenesis of EAU. Recipients of spleen cells cultured with Con A developed severe EAU along with T-cell mediated DTH immune responses, but without any detectable levels of antibodies. Furthermore, experiments with the fractionated subsets of T-lymphocytes demonstrated that the uveitogenic capacity localized with the helper/inducer subset. This finding is in line with reports showing that the helper/inducer subset of T-lymphocytes plays the main role in the adoptive transfer of EAE, 23,24 or allograft rejection. 25

The histologic changes in the recipient rats closely resemble those in rats actively immunized with S-antigen and CFA. These changes are typical of their “hyperacute” pattern (see references 1 and 26) and lack of chronicity, as manifested by the prevalence of PMNs and virtual absence of granulomatous reactivity. Intense PMN involvement in immunopathogenic reactions has been generally attributed to antibody-mediated Arthus-like responses and a similar assumption has been made concerning the prevalence of PMNs in ocular tissues in animals with EAU.1,7,26 However, our data show that this is not necessarily the case in the rat eye, since PMNs infiltration was found in eyes of recipients in which no antibodies to S-antigen could be detected, namely, recipients of spleen cells cultured with Con A, or the helper/inducer subset of these cells. The mechanism that causes the accumulation of PMNs at the early stages of EAU is not clear. We have suggested elsewhere 21 that an early injury to the rat eye releases a certain component that is highly chemotactic for PMNs. The assumption that the PMN involvement in the rat eye is mediated at least in part by a nonimmune mechanism is in accord with histologic findings in rat pineal glands. The rat pineal gland contains S-antigen 21 and undergoes inflammatory changes in animals in which EAU is induced by either active immunization or adoptive transfer of cells. 24 Unlike in the eye, however, only mononuclear infiltration was found in pineal glands of the affected rats. 21

Although PMN involvement has been found in recipient rats with no detectable antibodies to S-antigen, the proportion of PMNs in eyes of these rats was found to be somewhat lower than that observed in recipients with antibodies or in rats actively immunized with S-antigen (Table 3). It is conceivable, therefore, that the PMN infiltration in eyes of such rats may derive in part from Arthus-like responses, mediated by these antibodies.

The results reported here thus provide new insight into the pathogenesis of EAU. Three findings are of particular interest: (1) T-lymphocytes sensitized to S-antigen are capable of producing EAU, even in the absence of specific antibodies. It is possible, however,
that antibodies play an accessory role in the uveitogenic process. (2) T-lymphocytes of the helper/inducer subset play the major uveitogenic role. On the other hand, cytotoxic lymphocytes were found incapable of inducing EAU and probably have minimal or no role in producing the disease. It is further assumed that lymphokines produced by the helper/inducer lymphocytes are responsible for bringing about the inflammatory cell infiltration. (3) The inflammatory infiltration in eyes of recipient rats conspicuously consists of a large proportion of PMNs, even when no antibody-mediated mechanisms are involved. This finding underscores the uniqueness of the immunopathogenic mechanisms of EAU in the rat. More study is needed to further elucidate these mechanisms.

**Key words:** retinal soluble antigen (S-antigen), experimental autoimmune uveoretinitis (EAU), adoptive transfer of EAU, helper and suppressor lymphocytes

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**References**