Dynamics of Experimental Autoimmune Uveoretinitis Induced by Adoptive Transfer of S-Antigen-Specific T Cell Line

Chi-Chao Chan, Rachel R. Caspi, Francois G. Roberge, and Robert B. Nussenblatt

Long-term S-antigen (S-Ag) specific T lymphocyte lines can induce experimental autoimmune uveoretinitis (EAU) when transferred into naive rats systemically or intravitreally. The uveitogenic lymphocyte line (ThS) stains positively for the ART 18 (IL-2 receptor) and W3/25 (T helper/inducer); negatively for OX6 (RT1B) and OX8 (T suppressor/cytotoxic cells). The inflammation induced by systemic or intravitreal transfer of the ThS line was studied with respect to the surface markers of the infiltrating cells, as well as the markers expressed by the resident ocular cells, by immunohistopathological techniques. On day 4 to 5 after systemic injection of the ThS line, rare T cells (W3/25+, ART 18+) and macrophages (OX42+, OX6+) surrounded by some resident cells that expressed MHC class II antigens were identified in the ciliary body and choroid. Shortly thereafter (10–20 hr) more macrophages, polymorphonuclear leukocytes, and T lymphocytes (W3/25+, then OX8+) appeared in the inflamed eye. The kinetics was similar to EAU induced by active immunization. The rats with severe disease expressed the MHC class II antigens on large numbers of resident cells in the eye. Intravitreally transferred ThS cells migrated to the retina within 24 hr of transfer. Infiltration of macrophages (OX6+, OX42+) and other T lymphocytes (OX6+, W3/25+ or OX8+), in conjunction with photoreceptor damage, were observed within the next 24–48 hr. The cells with the ThS markers disappeared from the eye on day 4–5 post transfer. These findings suggest that the ThS line can recognize the photoreceptor S-Ag in vivo. Destruction of the photoreceptors and the expression of MHC class II antigens by ocular resident cells may function in the recruitment and attraction of inflammatory cells elsewhere into the eye, and complete the process of EAU.

Experimental autoimmune uveoretinitis (EAU) is an intraocular delayed-type hypersensitivity (DTH)-associated inflammatory disease that can be induced in various animals by immunization with several different retinal proteins.1,2 One of the better known and well studied retinal antigens is the S-antigen (S-Ag), a 48 kd soluble protein located in outer segments of photoreceptors in the retina.3 Adoptive transfer of primed lymph node cells from S-Ag immunized rats,4 of long-term T cell lines derived from lymph node cells of S-Ag immunized rats,5 or of long-term S-Ag specific T helper/inducer cell lines (ThS)6 results in a similar ocular DTH inflammation which is clinically apparent 6–8 days post systemic transfer. The ThS line is uveitogenic when transferred into naive rats at 5 to 10 X 10^6 cells/rat systemically, or to 1 to 2 X 10^6 cells/rat intravitreally.6 The ThS line stains positively for ART18 (IL-2 receptor) and W3/25 (T helper/inducer), negatively for OX6 (RT1B) and OX8 (T suppressor/cytotoxic).6

This study was aimed at identifying the infiltrating cells which were recruited into the eye and evaluating the markers expressed by the ocular resident cells at various intervals during the latent period after the initiation of EAU induced by adoptive transfer of ThS line either systemically or intravitreally. The data obtained by immunohistochemical techniques provide insight into the dynamics of cellular changes which took place in the ocular immune-mediated inflammatory site. The data suggest that the ThS line recognizes the S-Ag in the photoreceptor region and precipitates photoreceptor damage possibly by recruiting the actual DTH effector cells from lymphoid organs to the target organ—the eye. Damage to the photoreceptor could attract additional cells into the inflammatory site and continue the EAU process.

Materials and Methods

Female Lewis rats, between 150–200 g (Charles River, Raleigh, NC) were the recipients for all experi-
ments. All procedures used in this study conform to the ARVO Resolution on the Use of Animals in Research.

Preparation of ThS Line

Long-term ThS lines were generated from the lymph nodes of S-Ag immunized Lewis rats as previously described. The cells used in all the experiments were obtained from aliquots of a frozen batch, which had been in continuous culture for over 2 months. For each experiment, an aliquot of cells was rapidly defrosted and resuspended in RPMI 1640 medium (Gibco, Grand Island, NY). Rat Con-A spleen supernatants were used as an IL-2 source. The cells were divided and then rested for 4 days before being restimulated with S-Ag (5 to 10 µg/ml) or with Con-A (5 µg/ml) on syngenic antigen presenting cells as described. The stimulated ThS cells were separated from antigen presenting cellular debris by density centrifugation over ficoll (Isolymph; Teva, Jerusalem, Israel), washed and counted prior to adoptive transfer.

Adoptive Transfer of ThS Line

Four separate experiments were performed. In each experiment, 12-20 rats received 10 x 10^6 ThS line cells systemically by intraperitoneal injection. Recipient rats were examined daily for the appearance of clinical EAU, and were killed on day 4, 5, 6, 7 or 8 after transfer. The eyes were removed for immunohistopathological evaluation. For the intravitreal adoptive transfer group, approximately 10^6 ThS line cells in a volume of 10 µl were injected through the pars plana into the right eye, after first emptying the anterior chamber to reduce intraocular pressure, as previously described. Ten milliliters of RPMI 1640 medium was injected in the same fashion into the left eye as a control. In a separate experiment, a ThP helper cell line (ThP), which is specific to purified protein derivative of tuberculin and nonuveitogenic, was injected into the vitreous of three rats using the same method as the intravitreal injection of ThS line described previously. The recipient rats were examined daily for clinical manifestation of EAU and were killed on day 0, 1, 2, 3 or 4 after transfer. The three rats injected with ThP were killed on day 1, 2 or 3 after transfer. As before, eyes were removed and studied by immunohistochemical techniques.

Immunopathology

Freshly enucleated eyes were immediately embedded in OCT (Miles Lab., Naperville, IL), snap frozen in a mixture of dry ice and methyl butane (−75°C) and stored at −70°C. The immunohistochemical technique was described previously. Six micrometer serial frozen sections were cut and placed on gelatinized slides. These slides were placed in 4°C for 48 hr prior to stain. After brief fixation in acetone, the sections were stained by avidin-biotin complex immunoperoxidase method as follows: mouse hybridoma-derived monoclonal antibodies against rat macrophages (OX42), T lymphocytes ((T helper/inducers (W3/25), T suppressor/cytotoxic cells (OX8)), IL-2 receptor (ART18) and RT1B (OX6, IA in mouse), RT1D (OX17, IE in mouse), as well as mouse ascitic fluid were the primary antibodies at a concentration of 1–2 µg/ml. After 1 hr incubation in a moist chamber at room temperature, the primary antibodies were washed. The secondary antibody, biotin-labelled goat anti-mouse IgG, that was depleted of antibody cross-reactivity with rat IgG (American Qualex Inc., La Mirada, CA, diluted 1:100) was applied. After another hour incubation, then washing, avidin-biotin-peroxidase complex (Vector Lab., Burlingame, CA) 1:100 was added for 45 min. Then the slides were washed and developed in the substrate of 3,3′ diaminobenzidine-NiSO₄-H₂O₂ solution.

Data Collection

Cells were considered positive, if they showed a dense, dark black-blush peripheral ring of stain on the cellular membrane. The positive cells that stained positively for each primary monoclonal antibody in different ocular tissue were counted and recorded in the same fashion as previous studies performed in active EAU.

Results

Systemic adoptive transfer of ThS line resulted in the development of clinical EAU on days 6–8 post transfer, and evidence of histopathological EAU on day 5 post transfer. Table 1 demonstrates the incidence of histopathological disease related to the time post transfer, as compiled from four separate experiments. On day 5 after adoptive transfer of ThS line, one-third of examined eyes demonstrated histopathological EAU. A higher incidence of histopathological EAU were observed between days 6–8 post adoptive transfer. The identification of different inflammatory cellular surface markers is summarized in Table 2. These data showed that on day 4, 1 day prior to the onset of histopathological EAU, a few cells with ART18⁺, W3/25⁺, OX42⁺ and OX6⁺ were identified in the ciliary body and choroid in only one examined rat. These cells suggested early infiltration of T helper lymphocytes (ART18⁺, W3/25⁺) and macrophages (OX42⁺, OX6⁺, some W3/25⁺). Most ocular resi-
Table 1. Incidence of EAU after systemic adoptive transfer of ThS lines.

<table>
<thead>
<tr>
<th>Days post transfer</th>
<th>Histopath. EAU/total examined eyes</th>
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<tbody>
<tr>
<td></td>
<td>4</td>
</tr>
<tr>
<td>Exp I</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>0/4</td>
</tr>
<tr>
<td>III</td>
<td>0/4</td>
</tr>
<tr>
<td>IV</td>
<td>0/4</td>
</tr>
<tr>
<td>Combined (%)</td>
<td>0/8 (0)</td>
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</tbody>
</table>

Table 2. Identification of different cellular surface markers after systemic transfer of ThS lines

<table>
<thead>
<tr>
<th>Days post transfer</th>
<th>Marker-positive cells/total histopath. EAU</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4</td>
</tr>
<tr>
<td>OX8*</td>
<td>–</td>
</tr>
<tr>
<td>OX6*</td>
<td>(1/0)±</td>
</tr>
<tr>
<td>OX42*</td>
<td>(1/0)±</td>
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* Severity of EAU, rated on a scale of - to ++++.
Macrophages (OX42+ , OX6+) and recruited T cells (W3/25+, OX8+) were observed in the uvea. Between days 2–3 the cells stained for ThP decreased and other recruited inflammatory cells increased. A few of the inflammatory cells could be identified in the inner retina; however, no specific photoreceptor involvement could be noted. Foci of ocular resident cells stained positively for MHC class II antigens.

Discussion

The present report demonstrates rapid, dynamic changes inside the eye during EAU induced by the adoptive transfer of ThS line introduced via either systemic or intravitreal route. Within 24 hr of intravitreal injection of the ThS line, these cells had migrated toward the retina, where S-Ag is located and had disappeared from the eye within 4 days. It is possible that these ThS line cells may lose their IL-2 receptors in vivo very rapidly. Immediately following the migrating pathway of the ThS line, other inflammatory cells of DTH reaction (macrophages, T helper/inducers, then T suppressor/cytotoxic cells) were recruited rapidly into the eye. The ability of the migration of ThS line toward the retina before other inflammatory cells infiltrated the eye and the induction of photoreceptor damage as compared to the similar migration of ThPPD without retinal damage may imply a direct role of ThS cells in the effector mechanism of EAU as suggested previously. Production of lymphotoxins by T helper cells in vitro had been described previously, and in experimental autoimmune encephalomyelitis (EAE), myelin-basic-protein-specific autoaggressive T helper cell lines can be directly cytotoxic in culture to antigen presenting organ—the resident glial cells. When present in sufficiently large numbers, as during direct intravitreal administration, the ThS cells might be able to directly damage the photoreceptor, even before the recruitment of the more classical DTH effector cells—the macrophages. No specific retention of the cells bearing markers typical of the ThS line was observed in these eyes, even after foci of photoreceptor damages developed. This result is similar to that seen by injecting the ThS line or naive lymph node cell using autoradiographic techniques.

The control eyes did not exhibit EAU induced by adoptive transfer within the time period studied, or even as late as 2 weeks post transfer. In the classical DTH, the T cell must recognize the specific antigen in the context of class II in order to react. Therefore, it could be possible that since ThS can recognize S-Ag in the photoreceptor and is stimulated in vitro just prior to intravitreal injection, the factors released by

Fig. 1. Day 1 after intravitreal injection of ThS line cells showed that majority of cells in the vitreous (V) stained positively for ART18 (a), negatively for OX6 (b). (R—retina; ABC-immunoperoxidase; original magnification ×125). Inset: Higher magnification showed the clump of ThS cells (original magnification ×312.5).
Fig. 2. Day 2 after intravitreal injection of ThS line cells showed that photoreceptor damage and cell infiltrates in the retina (R). Majority of infiltrating cells stained positively for W3/25 (a). Cells stained positively for OX42 were observed in the anterior vitreous (V) (b) (×125).

Fig. 2. (continued) Cells stained positively for ART18 were located in the posterior vitreous (V) (c). Majority of infiltrating cells stained negatively for OX8 (d). (ABC–immunoperoxidase; original magnification ×125).
this activated clone initiate either class II expression and/or the recruitment of other inflammatory cells. On the contrary, other nonuveitogenic T cell lines such as the ThP could not induce uveitis because they did not migrate to the photoreceptor well; their antigens were not presented in the context of class II, even though they could start recruitment and class II expression since they were also previously stimulated in vitro. Thus, induction of EAU by intravitreal transfer of activated T cells is restricted to the ThS line.

The progression of systemically transferred EAU, induced by the ThS line resembles the results of EAU induced by active immunization with S-Ag.8,9 Prior to histopathological EAU, on days 4–5 post transfer, a few infiltrating cells with ART18+, W3/25+ and OX42+ were found in the uvea (Fig. 1). At the same time, some ocular resident cells which surrounded or were near these infiltrating cells (such as vascular endothelia, fibroblasts and RPE) showed expression of MHC class II antigens. Shortly thereafter, more cells with W3/25+, OX42+ and OX6+, which most likely were macrophages, were recruited into the eye, in the uvea, retina, vitreous and anterior chamber. Then cells bearing the OX8 marker were also accumulated in the infiltrated ocular tissue. It is important to point out that in spite of the fact that the majority of infiltrating cells were T lymphocytes (W3/25+, OX8+, OX6+), only 10–20% of the infiltrating cells stained positively for IL-2 receptor (ART18+), and less than 10% of the infiltrating cells were negative for RT1B (OX6+). This observation may imply that only small numbers of infiltrating cells with the ThS line marker are involved throughout the entire EAU process. As suggested by Werdelin and McCluskey, the autoimmune reaction is initiated by the arrival to the site of a few specifically sensitized lymphocytes, probably on a random basis.15 Palestine and colleagues have shown that only a small number of the transferred rat indium labelled lymphocytes, obtained from draining lymph nodes of S-Ag immunized rats, reached the recipient eyes when adoptively transferred EAU was induced, but this number was greater than the accumulation of lymphocytes primed to another irrelevant antigen.16 A small but significant number of rat T lymphocyte lines, specifically against myelin basic protein, and functional in mediating EAE, was demonstrated to accumulate in the brain just before the onset of EAE.17

In this study, a very small number of cells with ART18+, W3/25+ (most likely ThS line cells) and OX42+ (macrophages) were detected in the eye before histopathological EAU occurred. These findings suggest that these S-Ag-specific cells recognize the S-Ag in the photoreceptors, following which soluble factors may be produced and released. These factors cause the influx of many more activated inflammatory cells (OX6+), macrophages (OX42+) and T lymphocytes (W3/25+ or OX8+) into the eye, resulting in amplification of the EAU process. Therefore, retention of ThS cells (ART18+ and OX6+) in the eye may not be necessary for the progression of EAU. These results are compatible to the study of the migration of adoptively transferred systemically 51Cr-labeled ThS and ThP.18 Low numbers of cells from the two lymphocyte lines were detected in the eyes of unprimed rats, with no obvious increase of ThS over ThP, despite the induction of EAU in the recipient animals by the injection of ThS line.

It is interesting to note that unlike EAU induced by active transfer,10 most ocular resident cells (ciliary epithelia, RPE, vascular endothelia) did not express MHC class II antigens (RT1B and RT1D in rat) on day 4 after transfer, even when significant numbers of

### Table 3. Incidence of photoreceptor damage after intravitreal adoptive transfer of ThS lines

<table>
<thead>
<tr>
<th>Days post transfer</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>combined (%)</td>
<td>0/4 (0%)</td>
<td>2/9 (22%)</td>
<td>7/9 (78%)</td>
<td>4/4 (100%)</td>
<td>2/2 (100%)</td>
</tr>
</tbody>
</table>

### Table 4. Dynamics of infiltrating cellular subtypes after intravitreal adoptive transfer of ThS line

<table>
<thead>
<tr>
<th>Percent of marker-positive cells/total infiltrating cells in the eye</th>
<th>Days post transfer</th>
</tr>
</thead>
<tbody>
<tr>
<td>ART18+</td>
<td>100%</td>
</tr>
<tr>
<td>W3/25+</td>
<td>100%</td>
</tr>
<tr>
<td>OX8+</td>
<td>0%</td>
</tr>
<tr>
<td>OX6+</td>
<td>0%</td>
</tr>
<tr>
<td>OX42+</td>
<td>0%</td>
</tr>
</tbody>
</table>
inflammatory cells reached the eye, or when histopathological EAU was apparent. Expression of class II antigens on the resident cells in the target organ prior to the inflammation has been hypothesized to play an important immunopathogenic role in the initiation and perpetuation of various organ-specific autoimmune processes, including EAU induced by active immunization of S-Ag. However, a number of observations have pointed to factors or conditions other than ones directly related to the initial induction of class II expression on organ resident cells in the initiation of some autoimmune disorders. For example, in Graves' disease, the correlation between follicular HLA-DR expression and local infiltration is incomplete: occasional follicles strongly express HLA-DR, despite a paucity of infiltrating lymphocytes; in type I diabetes, the majority of islets containing HLA-DR positive beta cells show no insulitis; conversely, in other diseases involving pancreatic infiltration, eg chronic pancreatitis and cystic fibrosis, no HLA-DR expression by the islet cells is observed. Thus, full expression of RT1B and RT1D on all ocular resident cells prior to EAU induced by adoptive transfer of ThS line might not be obligatory for initiating and triggering or even perpetuating the disease, and may represent a secondary phenomenon, resulting from a massive release of inflammatory mediators. The result of expression of class II antigens on ocular resident cells in the rats injected with ThP without initiation of EAU supports this hypothesis.

One of the principal modulating influences on MHC class II antigen expression in vitro and vivo is probably gamma interferon. In adoptive transfer, the latent period before expressing disease is much shorter (5 to 6 days) than that in active immunization of S-Ag (11 to 13 days). Therefore ocular resident cells may have a shorter duration to expose to local gamma interferon, which is produced by circulating autoreactive T lymphocytes. Other influences on MHC class II antigen expression include noninterferon interleukin, adjutants, release of autoantigen from damaged tissues, etc. In adoptive transfer, the intensity of ocular inflammation is milder, and shows mild involvement of the anterior chamber and lower titers of serum anti-S-Ag antibodies. On the other hand adjutants are required for active immunization. Thus, lower RT1B and RT1D expression on ocular resident cells may be observed in adoptive transfer. Whether class II expression plays a key role in the propagation and initiation of EAU, or is more probably a secondary phenomenon which simply indicates that the pathogenetic mechanism involves T lymphocytes, requires further investigation.

**Key words:** immunoperoxidase technique, experimental autoimmune uveoretinitis (EAU), adoptive transfer, S-antigen (S-Ag), T lymphocyte line, inflammatory cellular markers

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**References**


