Suppression of Experimental Uveitis in Rats by Anti-I-A Antibodies

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I-region–associated (Ia) class II major histocompatibility complex (MHC) products are known to play a major role in autoimmunity. Effects of anti-I-A and anti-I-E monoclonal antibodies on development of experimental autoimmune uveitis (EAU) were investigated in Lewis rats. Prior to sensitization with S-antigen, seven groups of rats, six in each group, were injected intraperitoneally with one of the following agents. Groups 1, 2, and 3 (controls) received saline, RPMI and mouse immunoglobulin G (IgG), respectively. Groups 4 and 5 were injected with anti-I-E antibodies, 80 ng and 1000 ng, respectively. Similarly, groups 6 and 7 received anti-I-A, 100 µg and 750 µg, respectively. The treatments were repeated on days 1, 2, 5, 8, and 11 after S-antigen injection. All these animals were killed on day 18. In addition, two groups of rats sensitized with S-antigen were treated with 750 ng anti-I-A antibodies on days 5, 6, and 7 (group 8) and on days 7, 8 and 9 (group 9). An additional group (group 10) of Lewis rats was treated with 750 µg anti-I-A 1 day prior to and on days 1 and 2 after S-antigen injection. These group-10 animals were killed on day 31. Histopathologically, the enucleated globes of animals treated with high dose anti-I-A revealed marked suppression or inhibition of uveitis development. Such inhibition was virtually complete when the antibody was administered within a week of S-antigen injection, and the inhibitory effect lasted for at least 31 days. Three of six control animals with high dose anti-I-E antibodies showed inhibition of uveoretinitis. Many of the animals and those treated with low-dose anti-I-A and anti-I-E antibodies showed severe uveoretinitis. These results suggest that an I-A epitope plays a major role in the initiation of S-antigen-induced uveitis and that this intraocular inflammation can be suppressed by anti-I-A antibodies.

Administration of corticosteroids in various forms has been the mainstay in the management of uveitis. Although the vast majority of patients with uveitis show improvement with corticosteroids, some individuals with uveitis either fail to respond to steroids at maximum tolerable dose levels or develop various complications related to corticosteroids. Some of these patients benefit from cytotoxic drugs or cyclosporine A, but these drugs can also lead to serious side effects. The corticosteroids and cytotoxic agents control uveitis by nonspecific immune suppression. Cyclosporine A is a specific inhibitor of thymus-dependent lymphocyte (T-cell) function, and has been successfully utilized for treatment of T-cell–mediated intraocular inflammations in humans and of experimentally induced uveitis in laboratory animals.

Uveoretinitis induced by retinal S-antigen serves as a useful animal model for uveitis, and in Lewis rats this ocular inflammation has been shown to be mediated by helper/inducer T-cells. Helper/inducer T-cells express antigenic molecules, known as CD4, on their surfaces. These surface molecules are localized near the site of T-cell receptors that recognize I-A antigen. It has been shown that anti-CD4 antibodies can block in vitro antigen-driven activation of antigen-specific T-cell clones, and can prevent in vivo development of experimental autoimmune disease. Helper/inducer T-cells recognize antigens that are presented by macrophages or other antigen-presenting cells (APC) in context with major histocompatibility complex (MHC) class II gene products, known as I-region–associated (Ia) antigens. The two loci in the rat I region of the MHC, I-A and I-E, are homologous to HLA-DQ and HLA-DR in humans. In vivo administration of monoclonal antibodies specific for Ia antigens has been shown to inhibit and suppress a
number of experimental autoimmune diseases.\textsuperscript{8-13} Therefore, we hypothesized that it is possible to inhibit the development of autoimmunity and experimental uveitis by in vivo administration of monoclonal antibodies directed against I-A molecules.

During preparation of this manuscript we became aware of a recent report describing suppression of experimental uveoretinitis by a single injection of anti-I-A antibody.\textsuperscript{14} In the present study, we report the effects of treatment with varying doses of anti-I-A and anti-I-E antibody at different time periods after S-antigen injection in the development of uveoretinitis.

**Materials and Methods**

Initially, 42 Lewis rats weighing about 175 g each were sensitized with 50 μg of retinal S-antigen in complete Freund's adjuvant containing 2 mg/ml heat-killed tubercle bacilli (Difco, Detroit, MI). The S-antigen was isolated from bovine retina according to the method described by Dorey et al.\textsuperscript{15} Each animal received 0.2 ml of the suspension containing S-antigen and complete Freund's adjuvant in the foot pads. These animals were divided into seven groups of six each. Groups 1 through 3 served as controls. Group 1 was injected with normal saline, group 2 with culture media (RPMI-1640), and group 3 with 1000 μg of mouse immunoglobulin G (IgG), all in volumes of 0.5 ml per rat. These agents were injected intraperitoneally, 1 day prior to, and on days 1, 2, 5, 8, and 11 after S-antigen injection. At these same time points, groups 4 and 5 were injected with anti-I-E (OX 17, a mouse monoclonal IgG) monoclonal antibody, 80 μg and 1000 μg, respectively; groups 6 and 7 received anti-I-A (OX 6, a mouse monoclonal IgG), 100 μg and 750 μg, respectively (Table 1). This regimen was selected on the basis of a previous report on in vivo treatment of experimental allergic encephalomyelitis.\textsuperscript{16} Beginning 1 wk after S-antigen injection, the animals were examined every day for perilimbal conjunctival hyperemia and anterior chamber haziness. On day 18, under pentobarbital anesthesia, blood samples were obtained to detect anti-S antibody titers, as described by Mochizuki et al.\textsuperscript{14} The rats were then killed. The eyes were enucleated, and the right globes were fixed in 4% formaldehyde solution and submitted for histologic examination; the left eyes were snap-frozen for immunohistochemical studies. Two spleens, randomly selected from each group, were individually processed for splenic tissue lymphocyte transformation assay in the presence of S-antigen, as described previously.\textsuperscript{17} In addition, randomly selected spleens from each group were snap-frozen for immunohistochemical staining to identify I-A, I-E, CD4, and CD8 positive cells by an indirect immunoperoxidase technique utilizing appropriate primary and secondary antibodies, as described below. Similar immunoperoxidase staining was carried out on cryostat sections of the eyes. Sections of kidneys were processed for histopathologic studies to determine glomerular damage.

36 additional Lewis rats sensitized with 50 μg of S-antigen were divided into three groups. In group 8, six rats received 750 μg of anti-I-A antibodies on days 5, 6, and 7, and in group 9, six animals received the monoclonal antibodies on days 7, 8, and 9 after antigen injection. In group 10, six animals were treated with the monoclonals 1 day prior to and on days 1 and 2 after S-antigen injection. In each group, six additional rats served as controls and were injected with RPMI in the same manner as the experimental animals in groups 8, 9, and 10. Animals in groups 8 and 9, along with the corresponding control animals, were killed on day 21; animals in group 10, and their controls, were killed on day 31 after S-antigen injection (Table 2). The globes were enucleated and processed for histologic examination. All procedures were performed in accordance with the ARVO Resolution on the Use of Animals in Research.

**Detection of Antibodies to S-Antigen**

Blood samples were collected and analyzed by enzyme-linked immunosorbent assay (ELISA).\textsuperscript{4} 96-well polyvinylchloride plates (Dynatech Laboratories, Inc, Alexandria, VA) were coated with S-antigen (50 ng in carbonate buffer per well). After 1 hr incubation at 37°C, plates were washed five times with phosphate buffered saline (PBS) containing 0.05% Tween 20 (Sigma, St. Louis, MO). The wells were then incubated with PBS-Tween-20 at room temperature for 30 min. Diluted serum samples (100 μl) were added

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**Table 1. Decreased incidence of uveo-retinitis with anti-MHC class II antibody administration**

<table>
<thead>
<tr>
<th>Group*</th>
<th>Dosage† per rat</th>
<th>Number of animals‡ showing uveo-retinitis (Number out of 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Saline</td>
<td>0.5 ml</td>
<td>5</td>
</tr>
<tr>
<td>2. RPMI</td>
<td>0.5 ml</td>
<td>5</td>
</tr>
<tr>
<td>3. IgG</td>
<td>1000 μg</td>
<td>6</td>
</tr>
<tr>
<td>4. Anti-I-E (OX 17)</td>
<td>80 μg</td>
<td>6</td>
</tr>
<tr>
<td>5. Anti-I-E (OX 17)</td>
<td>1000 μg</td>
<td>3</td>
</tr>
<tr>
<td>6. Anti-I-A (OX 6)</td>
<td>100 μg</td>
<td>4</td>
</tr>
<tr>
<td>7. Anti-I-A (OX 6)</td>
<td>750 μg</td>
<td>1</td>
</tr>
</tbody>
</table>

* All groups were sensitized with 50 μg of S-antigen per animal, injected in complete Freund's adjuvant in the hind foot pad.
† All groups received intraperitoneal injections in a volume of 0.5 ml the day prior to and on day 1 and 2 after S-antigen injection. Treatment was repeated on days 5, 8, and 11.
‡ Enucleated eyes were processed and examined histologically.
after the wells were washed again. Following incubation for 1 hr at 37°C, plates were washed five times with PBS–Tween. 100 μl of peroxidase-conjugated anti-rat IgG (Dako Corporation, Santa Barbara, CA) in a dilution of 1:6000 was then added to each well, after which the plates were incubated at room temperature for 1 hr. After washing the plates, 100 μl of the substrate O-phenyl endiamine (Sigma) in citrate buffer containing 3% H2O2 was added to the wells. The reaction product developed in 15 to 30 min and was measured by absorbance at 490 nm using an automated device (Microelisa, Minireader MR590, Dynatech, Santa Monica, CA). Antibody levels were expressed as the absorbance values at 1:400 dilution of each serum sample.

**Lymphocyte Proliferation Assay**

Rat mononuclear cells were obtained by Ficoll-Paque (Pharmacia, Piscataway, NJ) centrifugation of dissociated spleen preparations. Proliferation assays were performed with RPMI-1640, 10% fetal calf serum, and 5 × 10⁻⁵ M 2-mercaptoethanol in flat-bottomed 96-well culture plates (Costar, Cambridge, MA). One hundred μl of mononuclear cell suspension (10⁶ cells/ml) was dispensed into each well, followed by 100 μl of appropriately diluted S-antigen, mitogen, or control reagents. Mitogen cultures were incubated for 3 days and antigen cultures were incubated for 6 days, both at 37°C. At the end of the culture periods, 1.0 μCi of ³H-thymidine was added to each well, the cultures harvested, and incorporated counts assessed. Thymidine uptake, in counts per min (CPM), was expressed as stimulation index.

**Stimulation index**

\[
\text{Stimulation index} = \frac{\text{CPM with S-antigen (or mitogen)}}{\text{CPM with medium}}
\]

**Indirect Immunoperoxidase Technique**

Sections 8 μm thick were cut from frozen globes and pieces of spleens. Sections were fixed in 99.5% acetone (reagent grade, VWR, Cerritos, CA) for 10 min, air-dried, and rinsed with PBS, pH 7.3. Primary antibodies specific for I-A, I-E, and CD4 (undiluted) and for CD8 (diluted 1:100) were applied to tissue sections for 30 min. The slides were then washed with PBS and incubated with peroxidase-conjugated goat anti-mouse IgG, 1:50 (Tago Inc, Burlingame, CA) at room temperature for 30 min. The slides were washed and treated with aminothylcarbazol (AEC) substrate in 1% H2O2 to develop the color. Slides were mounted in glycerogel and coverslipped.

**Monoclonal Antibodies**

Hybridoma cell lines producing anti-I-A (OX 6) and anti-I-E (OX 17) were a gift from Dr. D. W. Mason, Cellular Immunology Unit, Sir William Dunn School of Pathology, Oxford University, Oxford, England. The anti-CD4 monoclonal antibody (W3/25)-producing cell line was originally provided by Dr. Allan Williams, Oxford University. Isotype switch variants of this hybridoma were produced by one of us (LS), and the IgGl was used in this study. Anti-CD8 (OX8) was obtained from Accurate Chemical Corp. (Westbury, NY). All antibody producing hybridomas were received in a concentration of approximately 5 × 10⁵/ml in growth medium (RPMI-1640 + 10% fetal calf serum + antibiotics). Upon receipt, the contents of the flasks were centrifuged and resuspended in 10 ml fresh medium. Every other day the cultures were split. After a large volume had been obtained, the contents of the flasks were centrifuged, resuspended in serum-free medium, and incubated for 48 hr; supernatants containing the monoclonal antibodies were then collected. Protein concentration of the supernatants was measured using the standard Biorad assay.¹⁸ The supernatants were concentrated using ultrafiltration with ym10 filters (Amicon Corp, Danvers, MA). Dialysis was performed using membrane tubing; molecular weight cutoff was 12–14 kD (Spectrum Industries, Los Angeles, CA). Immunoglobulins were sterilized by pas-
sage through 0.22 μm filters (VWR), aliquoted, and stored at −70°C prior to injection.

**Determination of the Antibody Specificity and Analysis of Cell Populations by Flow Cytometry**

Using a technique similar to that described by McMaster and Williams,19 specificity of the monoclonal antibodies was determined. Five × 10⁵ spleen mononuclear cells were incubated with 50 μl of the supernatant antibody. The cells were washed twice with 1.5 ml PBS containing 0.5% bovine serum albumin and resuspended in 50 μl of fluorescein-conjugated rabbit F(ab')2 anti-mouse IgG antibody, 25 μg/ml. After two additional washes, the cells were analyzed by flow cytometry (FACS IV, Becton Dickinson, Mountain View, CA). This analysis revealed the presence of 83% I-E-positive monocytes and 43% I-E-positive lymphocytes. There were 91.3% positive monocytes for I-A and 41.8% I-A-positive lymphocytes. There were 41% CD4-positive lymphocytes and 63% CD4-positive monocytes.

**Results**

On clinical examination, the control animals in groups 1–3 (treated with saline, RPMI, and mouse IgG) developed conjunctival hyperemia on day 13 after S-antigen injection. This was followed by the appearance of cellular exudate in the anterior chamber and haziness of the vitreous cavity in the majority of the animals within 48 hr. Similar clinical features were noted in animals treated with low-dose anti-I-E (group 4). Three animals in group 5 (high-dose anti-I-E) and four animals in group 6 (low-dose anti-I-A) also showed clinical features of intraocular inflammation. No animals in groups 7 (high-dose anti-I-A) showed evidence of cells in the anterior chamber. However, by histopathologic examination, one animal in group 7 did have mild intraocular inflammation in one eye.

Histopathologically, uveitis and retinitis were noted in five of the animals in group 1 and in five of the animals in group 2. This intraocular inflammation was present in all 12 animals in groups 3 and 4. Some animals in groups 5 and 6 also had uveoretinitis. These results are summarized in Table 1. All animals with intraocular inflammation had marked inflammatory cell infiltration in the iris, ciliary body (Fig. 1), choroid, and retina. The infiltrates consisted predominantly of polymorphonuclear leukocytes admixed with mononuclear cells and with only a few plasma cells. There was prominent perivascularitis as well as vasculitis of the retinal vessels. Serous detachments of the retina were present in several animals, in which destruction of the photoreceptor cell layer and loss of outer retinal layers were also observed (Fig. 2).

In group 7, one animal had mild iritis, cyclitis, and focal choroiditis without necrosis of the retinal layers. The remaining five animals from this group and all six experimental animals from group 10 had no evidence of intraocular inflammation (Tables 1 and 2). In these animals, the uveal tract and retina were normal (Figs. 3, 4). Histologic examination of the kidneys obtained from all animals, regardless of treatment group, revealed no evidence of glomerulonephritis; glomerular architecture and tubules were unremarkable.

All of the control animals (treated with RPMI) in groups 8, 9, and 10 developed severe, diffuse intraoe-
ular inflammation. There was severe pan uveitis and destruction of outer retinal layers. In contrast, animals treated with the monoclonal antibodies (groups 8 and 9) had only mild focal choroiditis and retinal perivasculitis (Fig. 5). Similar mild ocular inflammation was present in only one animal in group 8 and in four animals in group 9 (Table 2).

Anti-S antibody levels in the serum from animals in groups 2, 3, 5, and 7, and lymphocyte transformation stimulation index determined on two randomly selected animals from groups 2, 3, 5 and 7, are summarized in Tables 3 and 4. In the treated groups, the stimulation indices, as well as the antibody levels, were significantly diminished (as determined by utilizing the student t-test) as compared with those in the control groups.

Indirect immunohistochemical studies were done on spleens of rats from the different groups to ascertain the percentage of cells positive for I-A, I-E, CD4, and CD8 molecules. Large numbers of I-A-, I-E-, and CD4-positive cells were observed in control groups 1 and 3. Group 5 animals, injected with anti-I-E, showed markedly reduced numbers of I-E positive cells only; group 7, injected with anti-I-A, exhibited few I-A positive cells (Fig. 6). Relative numbers of cells positive for the CD4 and CD8 markers were unchanged.

Immunohistochemical staining of frozen sections of the globes and evaluation of uvea revealed the presence of I-A-, I-E-, and CD4-positive cells in control groups 2 and 3. In the treated animals (group 7), sections demonstrated no inflammation and no positive staining, except for occasional I-A- and I-E-positive cells in the choroidal vasculature. Similar results were noted in the uninflamed eyes of animals treated with anti-I-E (group 5). In animals treated
with anti-I-E (groups 4 and 5), the eyes with uveitis showed the presence of I-A-, I-E-, CD4-, and CD8-positive cells.

**Discussion**

The experimental results of this study clearly establish the effectiveness of immunotherapy in the prevention of S-antigen–induced experimental uveitis. Administration of monoclonal antibodies directed against MHC class II determinants markedly suppressed and in some animals inhibited completely the development of uveitis. This inhibition lasted for at least 31 days, at which time animals were sacrificed. Since uveitis is a self-contained disease, development of the condition after this time period is highly unlikely. The anti-I-A treatment at dose levels of 750 μg, begun prior to S-antigen injection, inhibited development of uveitis in 11 of 12 animals and markedly suppressed inflammation in the remaining one animal. Similarly, when these antibodies were administered 5–7 days after the antigen injection, uveitis inhibition was noted in five of six animals. In contrast, when the treatment was delayed for 7–9 days, four of six rats developed uveitis. The ocular inflammation was milder, however, in this group than in untreated animals. These results indicate that the same Ia-dependent cellular components that are blocked by the immunotherapy in the initial stages of the disease also have a role in the progression of inflammation. In order to inhibit the development of uveoretinitis, it appears that the monoclonal antibodies should be used early in the immunization process. Previously, Chan et al and Fujikawa et al demonstrated expression of Ia antigen in the various ocular resident cells 2–4 days prior to development of clinical and histopathologic evidence of uveoretinitis induced by S-antigen. In the present experiment, lack of inhibition of uveoretinitis when the anti-I-A antibody was administered on days 7–9 suggests that the site of anti-I-A treatment effect could be extraocular, such as at the level of antigen presentation in the lymphoid organs.

Immunotherapy has been successful in other experimentally induced autoimmune diseases. Experimental allergic encephalomyelitis is an autoimmune demyelinating disease induced by sensitization with myelin basic protein. This experimental disease was inhibited by treatment with either anti-I-A or anti-CD4 monoclonal antibodies. Moreover, severity

**Table 3. Effects of anti-I-A treatment on humoral response**

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Anti-S antibody OD readings*</th>
<th>P values†</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>RPMI</td>
<td>1.74 ± 0.21</td>
<td>—</td>
</tr>
<tr>
<td>3</td>
<td>IgG</td>
<td>1.92 ± 0.06</td>
<td>—</td>
</tr>
<tr>
<td>5</td>
<td>Anti-I-E‡</td>
<td>0.74 ± 0.34</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>7</td>
<td>Anti-I-A§</td>
<td>0.25 ± 0.11</td>
<td>&lt;0.0005</td>
</tr>
</tbody>
</table>

* Anti-S response determined by ELISA. Results are expressed as mean absorption readings at 490 nm.
† Student t-test (two-tailed).
‡ Anti-I-E was injected in a dose of 1000 μg/injection.
§ Anti-I-A was used in a dose of 750 μg/injection.

**Table 4. Effect of anti-MHC class II treatment on the in vitro proliferation response to S-antigen and Con A**

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>S-antigen</th>
<th>Con A</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>RPMI</td>
<td>9.0 ± 0.12</td>
<td>39.9 ± 0.06</td>
</tr>
<tr>
<td>3</td>
<td>IgG</td>
<td>8.7 ± 0.08</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Anti-I-E</td>
<td>1.1 ± 0.32</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>7</td>
<td>Anti-I-A</td>
<td>1.1 ± 0.09</td>
<td>P &lt; 0.001</td>
</tr>
</tbody>
</table>

* Spleen cells from animals not sensitized to S-antigen revealed a mean stimulation index of 0.8. S-antigen was used with an amount of 27 μg/ml while Con A concentration was 5 μg/ml. The plates were incubated for 6 and 3 days respectively.
† Results obtained from animals that did not develop the disease. The proliferative response was not conducted from the animals which developed uveitis.
of the disease and morbidity from demyelinating inflammation were minimized by treatment with either of these antibodies, even after the development of signs of the demyelinating process. Similar beneficial effects were noted in the treatment of other experimentally induced or spontaneously developed autoimmune diseases affecting thyroid glands, kidneys, skeletal muscles, and joints.\(^5\) In a recent study of experimental autoimmune uveitis, anti-I-a treatment was effective in diminishing the disease.\(^14\)

The MHC gene products serving as restriction elements for helper or proliferating T-cells are the I-A and I-E glycoproteins. It is now established that different antigens are recognized by T-cells in association with distinct sites on the I-A or I-E molecule.\(^23\) Treatment with high doses of monoclonal antibodies directed against I-A clearly resulted in inhibition of the development of uveitis in the majority of animals. Similar but less convincing results were noted in animals treated with high doses of monoclonal antibodies directed against I-E. In this group, three of six animals showed inhibition of uveitis. In the limited number of animals studied, inhibition may be related to the fact that the epitope recognized by the anti-I-A monoclonal antibody may play a major role in S-antigen presentation and uveitis development, or may be related to antibody isotype and affinity; this question remains to be investigated. The importance of immunoglobulin isotype in monoclonal antibody therapy of autoimmune disease has been recently discussed.\(^24\) The specificity of an antibody molecule plays a major role in its therapeutic potential, and is determined by the variable region of its heavy and light chains. It is the constant region of the heavy chain molecule that determines immunoglobulin isotype and its effector function. Isotype variants of monoclonal antibody will have identical specificity for the corresponding antigen, but due to different heavy chain molecules, all variants may not have the same beneficial effect in amelioration of autoimmune disease; some will be superior to others.

In the present experiment, total inhibition of uveitis was noted in about 92% of animals (11 of 12 animals; groups 7 and 10) when the anti-I-A antibody was administered at dose levels of 750 \(\mu\)g and repeated at least twice during the early phase of priming with S-antigen. At lower doses, the inhibition or suppression of intraocular inflammation was not as effective. Similarly, Wetzig et al reported some suppression of intraocular inflammation, but no inhibition, when 400 \(\mu\)g of anti-I-A antibody was administered as a single injection a day prior to priming with foot pad injection of S-antigen.\(^14\) This report and the results of the present experiment suggest that the development of uveitis can be inhibited by high doses of monoclonal antibodies administered during S-antigen priming. Other reports also indicate that inhibition of autoimmune inflammatory processes are dose dependent.\(^8\)\(^-\)\(^12\)\(^,\)\(^25\)

Successful immunotherapy may therefore depend on the selection of an appropriate monoclonal antibody according to affinity, isotype, and sufficient dosage.

The precise mechanism(s) for the suppression of autoimmune uveitis by monoclonal antibodies is not clear from the results of the present experiment. Studies of other experimental autoimmune inflammatory diseases suggest such possible mechanisms for inhibition as compensatory activation of suppressor cells that recognize antigen in context with class II MHC when the class II-MHC–restricted activation of CD4 (helper/inducer cells) is blocked. Further, as the anti-I-A therapy leads to inhibition of T-cell immunity, it may stimulate antigen-specific suppressor T-cell activity.\(^23\) In vitro studies have revealed that anti-I-A antibodies can interfere with T-cell–dependent B-cell activation, and prevent interactions between T-cells. These monoclonal antibodies also impair the antigen-presenting functions of macrophages and dendritic cells and induce suppressor cells.\(^22\)\(^,\)\(^23\) One or a combination of these mechanisms may be operative in suppression of uveitis.

In the present study, immunoperoxidase staining of the splenic tissue suggests a selective loss of cells bearing MHC class II antigens after the in vivo treatment with anti-I-A antibodies (Fig. 6). Whether these cells are actually depleted, or whether I-A molecules are merely masked by or internalized after interaction with the anti-I-A antibody, remains to be demonstrated. Recent reports have established that loss of antigenic marker does not necessarily mean cell depletion, but may represent only capping and shedding of the marker or blocking of this marker by the monoclonal antibody.\(^22\) Physical cell elimination in our experimental system was not necessary for successful immunotherapy. The lack of specific proliferative response to the S-antigen indicates a specific blocking of antigen responses and not an overall loss of reactive cells, since response to concanavalin A (Con A) was not suppressed to the same extent as was noted with S-antigen (Table 4).

The experimental animals treated with the monoclonal antibodies did not develop any toxic side effects; none of the treated animals developed anaphylaxis or damage to kidneys. Further studies should be conducted in other animals, including nonhuman primates, prior to any consideration of this monoclonal therapy in humans with uveitis unresponsive to conventional therapy.
Key words: autoimmune uveitis, immunotherapy, anti-I-A antibodies, suppression, class II MHC

References


