Cyclosporine-Induced Alterations of Humoral Response in Experimental Autoimmune Uveitis

Chi-Chao Chon, Alan G. Palestine, and Robert B. Nussenblatt

Cyclosporine (CsA) has been shown to be effective in preventing S-antigen (S-Ag) induced experimental autoimmune uveitis (EAU) in Lewis rats. Alterations in the humoral immune responses associated with CsA therapy are illustrated by lower peak and delayed production of circulating anti-S-Ag antibodies in a proportional relationship to the dose of CsA in EAU. Circulating immune complexes were not detected in rats with EAU or in rats treated with CsA and without disease. These findings further support the important role of T-cells in EAU and further demonstrate the effect of CsA on helper T-cells. The kinetics of antibody production by S-Ag-immunized rats appears altered by CsA. Invest Ophthalmol Vis Sci 25:867-870, 1984

Bovine S-Antigen (S-Ag), a soluble retinal protein, can induce experimental autoimmune uveitis (EAU), which can affect both the anterior and posterior ocular segments in lower mammals. The histopathology showed intensive acute and chronic inflammation in anterior chamber, choroid, retina, and vitreous. The induction and appearance of EAU can be inhibited successfully by treating the animals with Cyclosporine (CsA). The alterations of the cellular immune response induced in this inflammatory model with CsA include a modulation in the in vitro proliferative responses of lymphocytes from the lymph nodes and peripheral blood of S-Ag-immunized animals and the morphologic absence of active blastogenesis in the draining lymph nodes of S-Ag-immunized rats. In this report, we have examined the effects of CsA on the B-cell response in EAU, and whether circulating immune complexes could be correlated with the protected or diseased state.

Materials and Methods. Female Lewis rats, 150-200 g in weight, (M. A. Bio-Products; Walkersville, MD) were used for all experiments. All rats in these studies were immunized with a single footpad injection of 30 μg of bovine S-Ag emulsified (1:1) in complete Freund’s adjuvant (CFA, GIBCO; Grand Island, NY) supplemented with H37 RA Mycobacterium tuberculosis (Difco; Detroit, MI). The bovine S-Ag was prepared as reported elsewhere and kindly provided to us by Dr. Waldon B. Wacker (University of Louisville; Louisville, KY). Sera of all animals were obtained at weekly intervals after immunization as indicated in the text. CsA, a gift from Sandoz (Basel, Switzerland) was dissolved in pure olive oil. Twenty treated rats were divided into three groups, and received 3 mg, 1 mg, or 0.1 mg CsA daily by subcutaneous injections into the thigh beginning on day 0 of immunization until killed. Twelve control animals received daily olive oil injections alone (Table 1).
Table 1. Number and grouping of EAU in Lewis rat

<table>
<thead>
<tr>
<th>Days post-immunization</th>
<th>Control rats (12)</th>
<th>3 mg/day (12)</th>
<th>1 mg/day (4)</th>
<th>0.1 mg/day (4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 7</td>
<td>12</td>
<td>12</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Day 14</td>
<td>8*</td>
<td>8*</td>
<td>4*</td>
<td>4*</td>
</tr>
<tr>
<td>Day 21</td>
<td>4*</td>
<td>4*</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* These rats were killed.

**ELISA technique:** The ELISA (enzyme linked immunosorbent assay) was performed using the method as described by Rennard et al. Briefly, 96-well polystyrene plates were coated with 0.5 μg of S-Ag diluted with carbonate buffer per well, incubated overnight at 4°C and then washed. Serial dilutions of tested sera were placed into each well. After the plates were incubated for 1 hr at 37°C and washed three times, the peroxidase-conjugated antiserum was added, and incubation continued for another 1 hr at 37°C. Then, a substrate solution of O-phenylenediamine (Sigma; St. Louis, MO) was added. The plates were incubated for 1 hr. The titer was defined as the highest dilution well with a yellow color. All data were analyzed using a two-sided, unpaired t-test.

**Clq precipitation and Raji cell assays:** The Clq binding precipitation assay was performed using the method of Zuber as modified by Lawley. The percent binding of Clq was expressed as a ratio of final CPM divided by initial CPM for each tube. A percent binding greater than 10% is considered positive in this assay. The Raji cell assays were performed using the method of Hall. A standard control using aggregated rat IgG was used and the results are expressed as equivalent μg/ml aggregated rat globulin.

These investigations conformed to the ARVO Resolution on the Use of Animals in Research.

**Results.** As had been noted previously, clinical uveitis presented by proptotic globe, severe hypopyon or hemorrhage in the anterior chamber, and dull red reflex was noted in all control rats by days 11–13. By day 20, the uveitis showed some improvement. Histopathologically severe panophthalmitis including choroiditis, retinitis, and vasculitis were disclosed in the eight, S-Ag-immunized, non-CsA-treated rats (controls) killed on day 14 (Fig. 1A). None of the animals that were immunized and treated with 3 mg of CsA daily developed any inflammation based on clinical evaluation, and histopathologic examinations on day 14 (eight rats) or day 21 (four rats) (Fig. 1B). Animals treated with 1 or 0.1 mg of CsA daily developed mild to moderate ocular inflammation on day 14 (Fig. 1C) that was less severe than Figure 1A. All animals had bilateral symmetrical pathology in each group.

**Anti-S-Ag antibodies:** In rats not treated with CsA, circulating anti-S-Ag antibodies (IgG), determined by the ELISA technique, were detectable as early as day 7 after S-Ag immunization and the anti-S-Ag antibody in four control rats killed on day 21 were still peaking. In the 3 mg daily CsA treated 12 rats however, circulating anti-S-Ag antibodies were not detected on day 7, but detected on day 14 with higher titers occurring on day 21. In rats treated with 1 mg or 0.1 mg CsA daily, detectable anti-S-Ag antibodies was measured on day 7 and 14, with the higher peak on day 14 (Fig. 2). The peak antibody titers in the 3 mg daily CsA-treated animals were (P < 0.05) delayed significantly as compared with the control group. In addition, the levels of the circulating anti-S-Ag antibody were lower (P < 0.05) delayed as compared with the control group. The levels of the circulating anti-S-Ag antibody also were lower (P < 0.05) on day 14 in the CsA-treated groups. However, by day 21 both groups had similar levels of antibody titer (Fig. 2).

**Circulating immune complexes:** The levels of circulating immune complexes in sera from CsA-treated and control animals were compared by both Clq and Raji cell assays. Normal rats had a Clq binding of 5 ± 2%. Rats treated with CsA 3 mg daily had a binding of 6.6 ± 0.7% compared with 7 ± 1% for rats not treated with CsA. Normal rats had an equivalent aggregated IgG (AG) of 8.5 ± 4 μg/ml in the Raji cell assay. Rats treated with CsA 3 mg daily had an AG of 3.3 ± 2 μg/ml compared with 5.0 ± 1.1 μg/ml for rats not treated with CsA. No statistical differences by t-test were found at day 14 after immunization in both groups.

**Discussion.** CsA, an endecapeptide, has potent immunosuppressive actions, with specific anti-T-cell effects. This effect may be due to its influence on IL-2. In vitro, CsA has been shown to cause a marked decrease in IL-2, either through decreased synthesis or release from activated T-helper cells in human peripheral blood lymphocytes or mouse spleen cell cultures. This may, therefore, result in a decrease in the recruitment of other potentially immunoreactive cells.

The inhibitive effect of CsA on T-helper cells plays a role in the generation of the humoral response to soluble antigens and the recruitment of other immunoreactive cells. T-helper cells can interact with resting B-cells and induce activation. They also can interact with accessory cells in order to induce the production of B-cell growth factors and other mediators.
Fig. 1. Fourteen days after immunization with 30 μg S-Ag. A, Control rats show marked inflammation (arrows), edema, and disorganization of the retina choroid. The subretinal space(s) is filled with fibrinous material and inflammatory cells. (Hematoxylin and eosin, ×200). B, The rats treated with CsA 3 mg/day are free of inflammation and show normal structure. The retina is separated artificially from the pigmented epithelium. (Hematoxylin and eosin, ×200). C, The rats treated with CsA 1 mg/day show mild acute inflammatory cell infiltration (arrows) on the surface of the mild edematous retina. (Hematoxylin and eosin, ×200).
EFFECT OF CYCLOSPORINE ON ANTI-S-ANTIGEN ANTIBODIES

Fig. 2. Titers of circulating anti S-Ag antibodies in CsA-treated rats show lower and delayed peak compared with the control rats. (Vertical Scale Log base 10; error bars show one standard error of the mean).

that are indispensable for B-cell clonal expansion and maturation into antibody secreting cells. Our finding of the delayed increase of the anti-S-Ag antibody titers in different daily dose CsA-treated rats indicates an alteration of the B-cell immune response by CsA. This alteration also shows an inversely proportional relation to CsA dosage. CsA appears to have changed the kinetics of humoral production, which may be caused by its effect on the recruitment of immune cells into sites of antigen processing. This probably is the result of CsA's effect on T-helper cells, although a primary effect on B-cells is not ruled out in this study.

Circulating immune complexes have been hypothesized as a compensatory (protective) mechanism accompanying retinal autoimmunity in patients. However, in our experiments circulating immune complexes were not detected in control or CsA-treated animals. These data would suggest that circulating immune complexes may not play a protective role in S-Ag-immunized animals treated with CsA. Also, there is no clear evidence in humans that circulating immune complexes (S-Ag and S-antibody complexes) play a role in the development of vasculitis. Our findings are consistent with the well-established selectivity of CsA toward T-cells and the primary role of T-cells in the production of EAU.

Key words: cyclosporine, S-antigen, experimental autoimmune uveitis, ELISA, circulating immune complex

From the National Institutes of Health, National Eye Institute, Bethesda, Maryland. Submitted for publication: October 11, 1983. Reprint requests: Chi-Chao Chan, MD, Clinical Ophthalmic Immunology Section, National Eye Institute, National Institutes of Health, Bldg. 10, Rm. 10D19, Bethesda, MD 20205.

References