Suppression of Experimental Uveitis With Monoclonal Antibodies to ICAM-1 and LFA-1

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Purpose. Intercellular adhesion molecule-1 (ICAM-1), which is one of the ligands for lymphocyte function associated antigen-1 (LFA-1), plays an important role in immune responses. To examine whether ICAM-1 and LFA-1 are involved in the pathogenesis of experimental autoimmune uveoretinitis (EAU), the authors investigated the therapeutic effect of anti-ICAM-1 monoclonal antibody (mAb) 1A29 and anti-LFA-1 α chain mAb WT.1 on retinal soluble antigen (S-Ag) and Freund’s complete adjuvant (FCA)-induced EAU in rats.

Methods. After immunization with S-Ag and FCA, rats were intraperitoneally injected with a monoclonal antibody, anti-ICAM-1 mAb 1A29 or anti-LFA-1 α chain mAb or control Ab, at 1.0 mg/kg body weight, according to the treatment schedule. Inflammation was examined clinically and histologically. Proliferative responses of splenocytes to S-Ag were also examined.

Results. The development of EAU could be completely prevented by the administration of 1A29, 1.0 mg/kg, twice a week from day 0 to day 14, but was only partially suppressed by WT.1. However, semiweekly administration of 1A29 from day 0 to day 7, or from day 10 to day 17, did not suppress EAU.

Conclusions. These findings indicate that ICAM-1, LFA-1-dependent pathways are involved in the pathogenesis of EAU. In addition, these pathways seem to be required for both the induction and the development of this disease. Invest Ophthalmol Vis Sci. 1994;35:2626-2631.

Experimental autoimmune uveoretinitis (EAU) has been widely used as a model of organ-specific autoimmune diseases. Retinal soluble antigen (S-Ag), a protein with a molecular weight of about 48 kilodalton (kDa), has been most extensively studied in relation to EAU. In recent years, it has been reported that adhesion of lymphocytes to various types of cells, including vascular endothelial cells, is important for immune response, indicating that adhesion molecules are involved in the pathogenesis of inflammation. ICAM-1, a 76-114 kDa glycoprotein belonging to the immunoglobulin supergene family, is located mainly in vascular endothelial cells and epithelial cells. LFA-1, located in peripheral lymphocytes, is a ligand for ICAM-1 and belongs to the integrin family. This ICAM-1/LFA-1 adhesion pathway is important in various cell-cell interactions and has been suggested to play a central role in the generation of the immune response. High expression of ICAM-1 at inflammatory sites and endothelial cells is induced by several cytokines, including interferon-γ, interleukin-1, and tumor necrosis factor. Recently, Iigo and colleagues reported the suppression of rat adjuvant arthritis by the administration of anti-ICAM-1 monoclonal antibody. To determine whether these molecules are involved in the autoimmune responses in EAU, we investigated the effects of anti-ICAM-1 and anti LFA-1 α chain monoclonal antibodies on the development of EAU in rats.

MATERIALS AND METHODS

Animals

Male Lewis rats, 6 weeks old, were purchased from Charles River Japan, Inc. (Atsugi, Japan). They were housed under standard pathogen-free conditions and were used for the study when they were 8 to 9 weeks of age.
age. The animals were treated in accordance with the ARVO Resolution on the Use of Animals in Research.

Antigen
S-Ag was purified as previously reported. Briefly, retinas removed from 100 bovine eyes were homogenized in 100 ml of 0.03 M phosphate-buffered saline and centrifuged at 12,000 rpm for 30 minutes. The yielded supernatant was fractionated by gel filtration with Sephacryl S-200HR (Pharmacia, Uppsala, Sweden), to collect fractions reactive to the rabbit anti-bovine S-Ag. The collected extract was further purified through a column packed with hydroxyapatite-agarose gel (Bio Rad, Richmond, CA).

Antibodies
The anti-rat ICAM-1 monoclonal antibody (1A29, mouse IgG,) and anti-rat LFA-1 α chain monoclonal antibody (WT.1, mouse IgG2a) used in this study have been well-characterized previously. Mouse IgG1 and IgG2a, (Becton Dickinson Immunocytometry Systems, Lincoln Park, NJ) were used as control antibodies.

Immunization
The rats were immunized by injecting 50 μg of S-Ag emulsified in Freund’s complete adjuvant (Difco Laboratories, Detroit, MI), containing 2.5 mg/ml of Mycobacterium tuberculosis H37Ra, at a ratio of 1:1, into the hind footpad. At the same time, 10¹⁰ nonviable cells of Bordetella pertussis (Wako Pure Chemical Industries, Ltd., Osaka, Japan) were injected intravenously.

Clinical and Pathologic Evaluation of EAU
After immunization, the rats were examined every day by slitlamp microscope by observers blinded to the treatment of individual rats. Inflammatory lesions of the eyes were classified as grade 0 to 4, according to severity, for five clinical signs: corneal edema, conjunctival hyperemia, ciliary injection of the cornea, anterior chamber inflammation, and posterior synchia. The scores of the five clinical signs were summed up and used as the clinical score. The clinical severity of each group was determined as the median of the clinical scores of individual rats. One animal of each group was killed on days 15, 18, and 21, and the eyeball was enucleated. These days were chosen to confirm the inhibition of EAU histologically based on preliminary experiments, in which EAU manifested on days 9 to 13 in almost all cases examined. Sections were stained with hematoxylin and eosin and were examined under a light microscope to confirm the onset of EAU histologically. Inflammation was graded from 0 (no inflammation) to 4 (panuveitis).

Antibody administration. In all experiments, each rat was intraperitoneally injected with an antibody at 1.0 mg/kg body weight according to the treatment schedules shown in Table 1. In the first set of experiments, four groups of five rats each were injected, twice a week, from day 0 to day 14 after inoculation with S-Ag, with anti-rat ICAM-1 monoclonal antibody (group 1), control IgG1 (group 2), anti-rat LFA-1 α chain monoclonal antibody (group 3), or control IgG2a (group 4), respectively; the remaining 10 rats received no antibodies (group 5). In the second set of experiments, four groups of five rats, or 20 rats in total, were given anti-rat ICAM-1 monoclonal antibody (group 6) or control IgG1 (group 7) on day 0, day 3, and day 7; or anti-rat ICAM-1 monoclonal antibody (group 8) or control IgG1 (group 9) on day 10, day 13, and day 17.

Lymphocyte Proliferative Response
Three rats each of groups 1, 3, and 5 were bled and killed on day 15 after S-Ag inoculation, the spleens were removed, and mononuclear cells were harvested. After two washings, 2 x 10⁶ mononuclear cells were suspended in 0.2 ml of RPMI 1640 (Nikken Biomedical Laboratory, Tokyo, Japan) supplemented with penicillin (100 μg/ml), streptomycin (100 μg/ml), L-

TABLE 1. Antibody Treatment Protocol

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>1A29</th>
<th>WT.1</th>
<th>Mouse IgG1</th>
<th>Mouse IgG2a</th>
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<tr>
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<td>5</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0, 3, 7, 10, 14</td>
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<td>5</td>
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<td>0, 3, 7, 10, 14</td>
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<td>5</td>
<td>—</td>
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<tr>
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<td>10</td>
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</tr>
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<td>0, 3, 7</td>
<td>—</td>
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</tr>
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<td>7</td>
<td>5</td>
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<td>0, 3, 7</td>
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</tr>
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<td>8</td>
<td>5</td>
<td>10, 14, 17</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>9</td>
<td>5</td>
<td>10, 14, 17</td>
<td>—</td>
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Anti-rat ICAM-1 mAb (mouse IgG1) (1A29), Anti-rat LFA-1 α chain mAb (mouse IgG2a) (WT.1), and nonspecific mouse IgG1 and IgG2a (Becton Dickinson Immunocytometry Systems), 1.0 mg/ml in PBS, were all given intraperitoneally at 1.0 mg/kg body weight twice a week.
glutamine (2 mM), and 5% inactivated fetal calf serum, together with S-Ag at various concentrations, followed by incubation in flat-bottomed 96-well plates (Becton Dickinson, Lincoln Park, NJ) at 37°C for 96 hours in 5% carbon dioxide. Six hours before the termination of incubation, [3H]thymidine (0.5 μCi/10 μl/well) (Amersham, Tokyo, Japan) was added. Cells were harvested, and incorporated 3H-TdR was measured with a liquid scintillator (Beckman Instruments, Fullerton, CA). Proliferative responses were expressed as mean ± SEM of stimulation indexes. In these experiments, a stimulation index of 2.5 or more was regarded as evidence of an in vitro "anamnestic" cellular response.

Evaluation Protocol

Fifty animals were kept under observation until day 28, and another 33 animals were killed in specific days for histologic examination or determination of proliferative response. The incidence, onset day, and clinical severity of EAU were evaluated in the 50 animals that were alive at the end of the study.

Statistical Analysis

Clinical scores were analyzed with the Mann-Whitney test, and other data were analyzed with the chi-square analysis and Welch's t-test on a two-tailed study. Values of P less than 0.05 were considered significant.

RESULTS

Effects of 1A29 and WT.1 on Development of EAU

Clinical time courses of EAU are shown in Figure 1. In the non-antibody-treated group (group 5), clinical evidence of EAU measured by clinical score was observed after about day 7, peaked at about day 14, and declined spontaneously thereafter. Control antibody-treated groups (groups 2 and 4), showed courses similar to group 5. In the WT.1-treated group (group 3), EAU was mild and its development was slightly delayed, although it was not statistically significant compared to the non-antibody-treated group (group 5). On the other hand, in the 1A29-treated group (group 1), the expression of EAU was markedly suppressed and the clinical score was significantly lower than the scores of the other groups (Fig. 1A). EAU occurred in only one of the five animals of group 1, on day 17. In group 5, 9 of the 10 animals developed EAU, and the incidence was significantly lower in group 1 than in group 5 (P < 0.05) (Table 2). However, group 3 showed no significant difference in incidence or severity from group 5 (Table 2).

Effects of 1A29 Administration in the Early and Late Phase of EAU

In the 1A29-early-treated group (group 6), although the development of EAU was slightly delayed, the degree of inflammation did not differ from the IgG1-early-treated control group (group 7). In the 1A29-late-treated group (group 8), EAU developed at the same time as it did in the IgG1-late-treated control group (group 9) with no difference in the degree of inflammation from group 9 (Fig. 1B). Thus, early or late administration of anti-ICAM-1 monoclonal antibody was insufficient for the prevention of EAU development (Table 2).

Histopathologic Examination

Histopathologically, eyes from the 1A29-treated group (group 1) had no inflammation or only mild inflammation (0 to 1+), characterized by focal cellular infiltration in the anterior chamber. In contrast, eyes from the group not treated with antibody (group 5) had extensive inflammation (4+), characterized by diffuse inflammatory cellular infiltration in the uvea, retina, vitreous cavity, and anterior chamber. In all groups, histologic severity was correlated with the clinical severity, as shown in Figure 1.

Effects of 1A29 and WT.1 on In Vitro S-Ag-Specific Proliferation of Splenocytes

Proliferative responses of splenocytes to S-Ag were compared among three groups. Mononuclear cells
TABLE 2. Incidence, Onset Day, and Clinical Severity of Uveitis

<table>
<thead>
<tr>
<th>Group</th>
<th>Antibody</th>
<th>Incidence*</th>
<th>Onset Day (mean ± SD)</th>
<th>Severity†</th>
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<tr>
<td>Experiment 1</td>
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<td></td>
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<td></td>
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<tr>
<td>1</td>
<td>1A29</td>
<td>1/5†</td>
<td>17</td>
<td>0§</td>
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<td>2</td>
<td>IgG1 control</td>
<td>5/5</td>
<td>9.1 ± 2.9</td>
<td>10 (7-13)</td>
</tr>
<tr>
<td>3</td>
<td>WT.1</td>
<td>5/5</td>
<td>11.1 ± 1.7</td>
<td>5 (3-7)</td>
</tr>
<tr>
<td>4</td>
<td>IgG2a control</td>
<td>5/5</td>
<td>9.5 ± 1.4</td>
<td>7 (5-10)</td>
</tr>
<tr>
<td>5</td>
<td>No treatment</td>
<td>9/10</td>
<td>9.9 ± 2.9</td>
<td>9 (0-12)</td>
</tr>
<tr>
<td>6</td>
<td>1A29 (early)</td>
<td>4/5</td>
<td>10.8 ± 3.3</td>
<td>6 (0-7)</td>
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<tr>
<td>7</td>
<td>IgG1 control (early)</td>
<td>5/5</td>
<td>10.6 ± 2.0</td>
<td>8 (2-10)</td>
</tr>
<tr>
<td>8</td>
<td>1A29 (late)</td>
<td>4/5</td>
<td>10.2 ± 0.8</td>
<td>7 (0-10)</td>
</tr>
<tr>
<td>9</td>
<td>IgG1 control (late)</td>
<td>5/5</td>
<td>10.2 ± 1.3</td>
<td>7 (2-14)</td>
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<table>
<thead>
<tr>
<th>Experiment 2†</th>
<th>Antibody</th>
<th>Incidence*</th>
<th>Onset Day (mean ± SD)</th>
<th>Severity†</th>
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<tbody>
<tr>
<td>1'</td>
<td>1A29</td>
<td>0/3†</td>
<td>—</td>
<td>0†</td>
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<tr>
<td>3'</td>
<td>WT.1</td>
<td>3/3</td>
<td>12.5 ± 3.2</td>
<td>6 (4-8)</td>
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<tr>
<td>5'</td>
<td>No treatment</td>
<td>5/5</td>
<td>9.9 ± 2.9</td>
<td>10 (7-12)</td>
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</table>

* Incidence was determined on day 28.
† Clinical severity of each group was determined as the median of clinical scores of rats on day 14.
‡ P < 0.01 compared with mouse IgG1 control (Group 2) (U test).
§ P < 0.01 compared with no treatment (Group 5) (x² test).
¶ This was an another experiment using the same protocol as experiment 1 to confirm the reproducibility of experiment 1.

The presence of lymphocytes strongly expressing LFA-1 adjacent to the ICAM-1 expressing cells suggests that the interaction of ICAM-1 with LFA-1 is important in the development of ocular inflammation in uveitis in humans. Antibodies against ICAM-1 and LFA-1 have been shown to reduce the severity of adjuvant arthritis,6 murine heterotopic corneal allograft rejection,9 murine cardiac allograft rejection,10 and rat experimental crescentic glomerulonephritis.11

Antibodies to cell surface molecules can affect cell function in several ways, including blockage or modulation of receptors, and signal transduction. Blockage of lymphoid or endothelial function by anti-ICAM-1, either through direct interference or by transduction of a negative signal, is the most plausible explanation for the results observed. Three sites of functional blockage can be considered: inhibition of sensitization, emigration, and target cell interaction. In this study, we showed that treatment of rats with anti-ICAM-1 mAb suppresses the ocular inflammation in EAU. Both clinical and histopathologic analyses revealed that anti-ICAM-1 mAb treatment for 2 weeks resulted in the suppression of EAU. The development of EAU can be divided into two phases, the induction phase and the effector phase, based on its pathogenesis: in the induction phase, T cells are stimulated by retinal S-Ag and uveitogenic T cells are generated; in the effector phase, inflammatory infiltration into the tissue is caused by the uveitogenic T cells. Whitcup and colleagues showed that IRBP-induced EAU using dif-
different mAbs in mouse was suppressed by daily intraperitoneal injections of anti-ICAM-1 or LFA-1 mAbs for 21 days. However, it was not clear from this experiment during which phase EAU development was suppressed. To elucidate the phase during which EAU development is suppressed by anti-ICAM-1 mAb treatment, the mAb was administered either during the induction phase (days 0 to 7) or during the effector phase (days 10 to 17). The suppressive effects of the mAb on EAU development could not be seen in either case, demonstrating that ICAM-1 was involved both in T cell sensitization and in consequent progression of uveitis.

On the other hand, in vitro proliferative response of splenocytes to S-Ag sensitized rats was suppressed by treatment with anti-ICAM-1 mAb for 2 weeks, which included both phases (Table 3). It is likely that the inhibitory effect of anti-ICAM-1 mAb reflects impaired production of uveitogenic lymphocytes in the induction phase. Iigo et al. reported the involvement of ICAM-1 in both the induction phase and the effector phase of adjuvant arthritis development, because the expression of adjuvant arthritis was inhibited by anti-ICAM-1 monoclonal antibody administered to either sensitized donors or naïve recipients before adoptive transfer. Taken together with our findings, it is conceivable that the inhibition of EAU by anti-ICAM-1 mAb was not primarily due to the blocking of only the interaction between antigen presenting cells and T cells, but to the blocking of both the induction and the effector phases of EAU development.

In a recent study, B10.A mice were immunized with IRBP and treated for 21 days with daily intraperitoneal injections of anti-ICAM-1 or anti-LFA-1 mAbs. Although clinical intraocular inflammation was significantly decreased in anti-ICAM-1 and anti-LFA-1 mAb treated animals, the histologic grade of inflammation was significantly decreased only in anti-ICAM-1 mAb treated mice compared to control mice. The discrepancy between the effects on EAU development of anti-ICAM-1 mAb treatment and anti-LFA-1 a chain mAb treatment has also been shown in EAU using the different mAbs in mice. Possible reasons for the difference between the two mAbs may be as follows. The first possibility is that the interaction of ICAM-1 in EAU induction is mediated by ligands other than LFA-1, such as Mac-1 (CD11b/CD18). Mac-1 is one of the polymorphonuclear cell (PMN) surface molecules, induced by activation, that mediate PMN adhesion to endothelial cells (ECs). When the expression of ICAM-1 is increased on cytokine-activated ECs, ICAM-1 provides a potential mechanism to enhance CD11b/CD18-mediated interaction, which makes PMNs rapidly adhesive to ECs. In addition to mononuclear cells, major pathologic involvement of PMNs has also been reported in the retina of EAU rats. A second possible reason is that greater concentrations of the anti-LFA-1 antibody were needed to block activated leukocytes. It was found that mAbs to ICAM-1 or LFA-1 alone were insufficient for prolonged tolerance of cardiac allografts, although it was also found that the two mAbs acted synergistically to inhibit graft rejection. The authors suggested that the redundancy of adhesion pairs may partly account for the synergism. ICAM-1 has other counter-receptors, Mac-1 and CD43. Mac-1 and LFA-1 bind to discrete domains on ICAM-1. However, the involvement of these adhesion molecules in immune reactions remains to be determined.

Key Words
ICAM-1 (intercellular adhesion molecule-1), LFA-1 (lymphocyte function associated antigen-1), experimental autoimmune uveoretinitis, S-antigen

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References


