The Complement System Plays a Critical Role in the Development of Experimental Autoimmune Anterior Uveitis

Purusbottom Jba,1 Jeong-Hyeon Sohn,2 Qin Xu,1 Hiroki Nishibori,2 Yali Wang,2 Saori Nishibori,2 Balasubramanian Manickam,1 Henry J. Kaplan,2 Puran S. Bora,1 and Nalini S. Bora1

PURPOSE. The role of complement in ocular autoimmunity was explored in an experimental autoimmune anterior uveitis (EAAU) animal model.

METHODS. EAAU was induced in Lewis rats by immunization with bovine melanin-associated antigen. Complement activation in the eye was monitored by Western blot for iC3b. The importance of complement to the development of EAAU was studied by comparing the course of intraocular inflammation in normal Lewis rats (complement-sufficient) with cobra venom factor–treated rats (complement-depleted). Eyes were harvested from both complement-sufficient and complement-depleted rats for mRNA and protein analysis for IFN-γ, IL-10, and interferon-inducible protein (IP)-10. Intracellular adhesion molecule (ICAM)-1 and leukocyte–endothelial cell adhesion molecule (LECAM)-1 were detected by immunofluorescent staining.

RESULTS. There was a correlation between ocular complement activation and disease progression in EAAU. The incidence, duration, and severity of disease were dramatically reduced after active immunization in complement-depleted rats. Complement depletion also completely suppressed adoptive transfer EAAU. The presence of complement was critical for local production of cytokines (IFN-γ and IL-10), chemokines (IP-10), and adhesion molecules (ICAM-1 and LECAM-1) during EAAU. Furthermore, intraocular complement activation, specifically iC3b production and engagement of complement receptor 3 (CR3), had a significant impact on disease activity in EAAU.

CONCLUSIONS. The study provided the novel finding that complement activation plays a critical role in the pathogenesis of ocular autoimmunity and may serve as a potential target for therapeutic intervention. (Invest Ophthalmol Vis Sci. 2006;47:1030–1038) DOI:10.1167/iovs.05-1062

From the 1Department of Ophthalmology, Jones Eye Institute, University of Arkansas for Medical Sciences, Little Rock, Arkansas; and the 2Department of Ophthalmology and Visual Sciences, Kentucky Lions Eye Center, University of Louisville, Louisville, Kentucky.

Supported in part by National Eye Institute Grants EY13335, EY014623, and R24 EY015656, the Commonwealth of Kentucky Research Challenge Trust Fund, and Research to Prevent Blindness, Inc.

Submitted for publication August 11, 2005; revised September 20, and November 9, 2005; accepted January 11, 2006.

Disclosure: P. Jha, None; J.-H. Sohn, None; Q. Xu, None; H. Nishihori, None; Y. Wang, None; S. Nishihori, None; B. Manickam, None; H.J. Kaplan, None; P.S. Bora, None; N.S. Bora, None

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be marked “advertisement” in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Corresponding author: Nalini S. Bora, Jones Eye Institute at University of Arkansas for Medical Sciences, 4301 West Markham, #523, Little Rock, AR 72205; nbora@uams.edu.

Complement is a major component of innate immunity. In recent years, it has become increasingly evident that complement is also involved in the antigen-specific immune responses with an identified role in antigen processing and presentation, T-cell proliferation and differentiation, B-cell activation, and systemic tolerance induced by the introduction of antigen into an immune-privileged site such as the anterior chamber of the eye. Inappropriate activation of complement has been implicated in various diseases, such as Alzheimer’s disease, ischemia–reperfusion injury, Huntington’s and prion disease, and multiple sclerosis (MS). However, the role of complement in ocular autoimmune disease is not well understood.

Experimental autoimmune anterior uveitis (EAAU) is an organ-specific autoimmune disease of the eye that serves as an animal model of idiopathic human anterior uveitis. We have previously shown that in this model, severe inflammation occurs in the anterior segment of the eye of Lewis rats after the footpad injection of melanin-associated antigen (MAA). Antigen-specific CD4+ T cells can adoptively transfer disease into naive syngeneic recipients and are the predominant inflammatory cells within the uvea. The purpose of the present study was to investigate the role of complement in the pathogenesis of EAAU.

MATERIALS AND METHODS

Animals

Male Lewis rats (5–6 weeks old) were obtained from Harlan Sprague-Dawley (Indianapolis, IN). All animals were treated in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Antibodies and Reagents

The IgG fraction of goat antisera to rat C3 was from ICN Biochemicals, Inc (Aurora, OH) and purified IgG fraction of rabbit anti-rat-irrterferon-inducible-irrteron protein (IP)-10 was from Torrey Pines Biologics, Inc. (Houston, TX). Purified rabbit IgG (polyclonal) immunoglobulin isotype standard, FITC-labeled mouse anti-rat CD54 (ICAM-1, IgG1), FITC-labeled hamster anti-rat CDE2L (selectin, leukocyte–endothelial cell adhesion molecule [LECAM-1], IgG2a) monoclonal antibodies, and respective isotype controls were purchased from BD Biosciences (San Diego, CA). Mouse anti-rat CD11b (MRC OX-42, IgG2a), FITC-labeled goat anti-mouse IgG2a (rat adsorbed) and isotype control antibodies were from Serotec (Raleigh, NC). Purified cobra venom factor (CVF) was from Quidel Corp. (San Diego, CA). Bovine serum albumin (BSA) and ITS+1 liquid medium supplement were from Sigma-Aldrich (St. Louis, MO).

Induction and Evaluation of EAAU

Lewis rats were immunized with 100 μL of stable emulsion containing 75 μg MAA emulsified (1:1) in complete Freund’s adjuvant (CFA). Difco...
Studies in a syngeneic rat model of EAAU showed that complement activation occurred within 3 days of immunization and was associated with inflammation. In this study, we examined the relationship between the intensity of ocular inflammation and complement activation in Lewis rats immunized with MAA, a common antigen used in the induction of EAAU. The intraocular tissues were prepared as described elsewhere. They comprised uvea, retina, aqueous humor, and vitreous and were used for total RNA extraction, ELISA, and Western blot analysis. Blood was collected by cardiac puncture, and total hemolytic complement activity in serum was determined using sensitized sheep erythrocytes (Diamedix, Miami, FL) according to the manufacturer’s directions. Serum from naive Lewis rat was used to determine the 100% value for complement-dependent serum hemolytic activity.

Adoptive Transfer of EAAU

Adoptive transfer of EAAU was performed as previously described by us. MAA-injected animals were divided into three groups, each containing five animals. Donor Lewis rats in group 3 received CVF at day 7 after immunization with MAA. Control animals received CVF 24 hours before the cell transfer. Animals in groups 1 and 3 received a similar injection of sterile PBS. Ten million cells were transferred by intraperitoneal injection. These experiments were repeated three times with similar results.

Table 1. Primer Sequences Used in RT-PCR

<table>
<thead>
<tr>
<th>Protein</th>
<th>Primer</th>
<th>Sequence</th>
<th>Amplified cDNA (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-Actin</td>
<td>Forward</td>
<td>GCC-CTC-CTC-GTC-AGA-AG</td>
<td>355</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>CTG-TTG-TGC-CAA-ATG-TTC</td>
<td></td>
</tr>
<tr>
<td>IL-10</td>
<td>Forward</td>
<td>AAG-GAC-CAG-CTG-GAG-AT</td>
<td>292</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>AGA-CAC-CTT-TGT-GAA-GCT</td>
<td></td>
</tr>
<tr>
<td>IFN-γ</td>
<td>Forward</td>
<td>ATG-TGG-AGG-AAC-TGG-CAA</td>
<td>288</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>GCT-TAG-GTA-AGT-TGG-TGA</td>
<td></td>
</tr>
<tr>
<td>IP-10</td>
<td>Forward</td>
<td>TGT-CTG-CAA-GTC-TAT</td>
<td>560</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>TTT-GCC-ATG-TCA-GTG-GGA</td>
<td></td>
</tr>
</tbody>
</table>

The sequences of all oligonucleotides are shown in the 5’ to 3’ direction.

Adoptive transfer of EAAU was performed as previously described by us.1,10-14 MAA-injected animals were divided into three groups, each containing five animals. Donor Lewis rats in group 3 received CVF at day 7 after immunization. Donor animals in groups 1 and 2 received a similar injection of sterile phosphate-buffered saline (PBS). Donor rats from each group were killed at day 11 after immunization and popliteal lymph nodes (LNs) were harvested separately. A single cell suspension of LN cells was prepared by digestion in Dulbecco’s modified essential medium (DMEM) and T cells were purified on immunocolumns (Cellect; Millenium Biologix Corp., Kingston, Ontario, Canada) according to the manufacturer’s directions. Purified T cells obtained from donor animals in group 1 were cultured in vitro with antigen for 3 days in serum-free DMEM supplemented with ITS+1 culture supplement before transfer to naive syngeneic host. Recipient animals in group 2 received CVF 24 hours before the cell transfer. Animals in groups 1 and 3 received a similar injection of sterile PBS. Ten million cells were transferred by intraperitoneal injection. These experiments were repeated three times with similar results.

Complement Depletion

To deplete complement in vivo, animals were divided into five groups. Lewis rats in each group received a single intraperitoneal injection of purified CVF (35 units, 500 μL) 24 hours before immunization or at day 4, 9, 14, or 19 after immunization with MAA. Control animals received 500 μL of sterile PBS. Total hemolytic complement activity in serum was determined as described earlier.

Sample Collection and Total Hemolytic Complement Activity

The intraocular tissues were prepared as described elsewhere. They comprised uvea, retina, aqueous humor, and vitreous and were used for total RNA extraction, ELISA, and Western blot analysis. Blood was collected by cardiac puncture, and total hemolytic complement activity in serum was determined using sensitized sheep erythrocytes (Diamedix, Miami, FL) according to the manufacturer’s directions. Serum obtained from naive Lewis rat was used to determine the 100% value for complement-dependent serum hemolytic activity.
Immunohistochemistry

Five-μm paraffin-embedded sections were immunostained with FITC-labeled monoclonal antibodies (1:100) directed against ICAM-1 and LECAM-1. Mouse anti-rat CD11b (1:200) and FITC-labeled goat anti-mouse IgG2a was used to stain complement receptor 3 (CR3) on the infiltrating cells. Control stains were performed with nonrelevant antibodies of the same immunoglobulin subclass at concentrations similar to those of the primary antibodies. Sections were examined under a fluorescence microscope (Carl Zeiss Meditec, Inc., Thornwood, NY).

Reverse Transcription–Polymerase Chain Reaction

Eyes were harvested from Lewis rats killed at different time points during EAAU. Intraocular tissue prepared as described earlier was pooled separately for each time point (n = 6 eyes/time point). Total RNA (0.1 μg) isolated from pooled intraocular tissue using SV total RNA isolation kit (Promega, Madison, WI) was used to detect the mRNA levels of β-actin, IFN-γ, IL-10, and IP-10 by semiquantitative RT-PCR, using reagents purchased from Applied Biosystems (Foster City, CA). The sense and antisense oligonucleotide primers (Table 1) were synthesized at Integrated DNA Technologies (Coralville, IA). Polymerase chain reaction was performed for 25 cycles. All reactions were normalized for β-actin expression. The negative controls consisted of omission of RNA template or reverse transcriptase from the reaction mixture.

Enzyme-Linked Immunosorbent Assay

Eyes were harvested from Lewis rats killed at different time points during EAAU. Intraocular tissue prepared as described earlier was pooled separately for each time point (n = 6 eyes/time point) and homogenized in 500 μL of ice-cold PBS containing protease inhibitors. After centrifugation, the supernatant was assayed (in triplicate) for rat IL-10 protein using rat ELISA kits from Biosource International (Camarillo, CA). Rat IFN-γ was assayed with a rat ELISA kit from R&D Systems.

Table 2. Effect of Complement Depletion on EAAU

<table>
<thead>
<tr>
<th>MAA (μg)</th>
<th>Eyes with EAAU</th>
<th>Duration of Disease (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Incidence</td>
<td>Mild</td>
</tr>
<tr>
<td>Control</td>
<td>100</td>
<td>30/30</td>
</tr>
<tr>
<td>CVF (day -1)</td>
<td>100</td>
<td>30/30</td>
</tr>
<tr>
<td>CVF (day 4)</td>
<td>100</td>
<td>30/30</td>
</tr>
<tr>
<td>CVF (day 9)</td>
<td>100</td>
<td>2/30</td>
</tr>
<tr>
<td>CVF (day 14)</td>
<td>100</td>
<td>30/30</td>
</tr>
<tr>
<td>CVF (day 19)</td>
<td>100</td>
<td>30/30</td>
</tr>
</tbody>
</table>

Incidence of EAAU given as positive/total eyes following clinical examination. Data are presented as the mean ± SD. Severity of inflammation on histopathologic examination was grouped as mild (1+), moderate (2+ to 3+) or severe (4+). This experiment was repeated three times (n = 5 rats/experiment) with similar results.

* P < 0.05.
systems, Inc. (Minneapolis, MN). Cytokine concentrations in the test samples were calculated by comparison with a corresponding standard curve, and all results were expressed as the mean concentration (picograms per milligram protein) of cytokine/SD. These experiments were repeated three times with similar results.

**Western Blot Analysis**

The sample was prepared as described for the ELISA. After SDS-PAGE on a 12% linear slab gel (10 μg total protein/lane), under reducing conditions, separated proteins were transferred to a polyvinylidene difluoride (PVDF) membrane.15 Blots were incubated with the IgG fraction of goat anti-rat C3 or the IgG fraction of rabbit anti-rat IP-10. Control blots were treated with the same dilution of goat or rabbit IgG isotype control, respectively. After washing and incubation with horse-radish peroxidase (HRP)-conjugated secondary antibody, blots were developed with an enhanced chemiluminescence Western blot analysis detection system (ECL Plus; GE Healthcare, Piscataway, NJ). Quantification of iC3b was accomplished by analyzing the intensity of the bands on computer (Quantity One 4.2.0; Bio-Rad Laboratories, Richmond, CA).

**In Vivo Antibody Treatment**

MAA injected Lewis rats (group 1, n = 5) received a single injection of 0.5 mg of mouse anti-rat CR3 (OX42) intraperitoneally daily, from day 9 to 14 after immunization. Control animals in groups 2 (n = 5) and 3 (n = 5) received a similar treatment with isotype matched monoclonal antibody and PBS alone, respectively.

**Statistical Analysis**

Differences between groups were evaluated by Student’s t-test. P < 0.05 was considered significant.

---

**FIGURE 3.** Effect of systemic complement depletion on intraocular IFN-γ, IL-10, and IP-10 mRNA expression during EAAU. Eyes (n = 6/time point) were harvested from MAA-injected, complement-sufficient (PBS-treated) and -depleted (CVF-treated) Lewis rats killed at different time points for semiquantitative RT-PCR analysis. PCR products were analyzed on a 2% agarose gel. (A) mRNA expression of IFN-γ and IL-10 in complement-sufficient, MAA-injected rats, and (B) represents mRNA expression of these cytokines in complement-depleted, MAA-injected rats. There was decreased production of IFN-γ and IL-10 mRNA in complement-depleted animals (B) compared with complement-sufficient rats (A). IP-10 mRNA expression was abolished by CVF treatment (C). A strong band at 335 bp for β-actin indicated an equal amount of RNA in each lane (A, B). Shown are ethidium-bromide–stained bands for PCR products after UV exposure. Images are representative of results in three independent experiments.

---

**TABLE 3.** Effect of Complement on the Adoptive Transfer EAAU

<table>
<thead>
<tr>
<th>Group</th>
<th>Donor Treatment</th>
<th>Recipient Treatment</th>
<th>Number of Cells (10⁶)</th>
<th>Cell Population</th>
<th>Incidence</th>
<th>Score</th>
<th>Day of Onset</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>None</td>
<td>None</td>
<td>10</td>
<td>T lymphocytes</td>
<td>30/30</td>
<td>Severe</td>
<td>7 ± 1</td>
</tr>
<tr>
<td>2</td>
<td>None</td>
<td>CVF</td>
<td>10</td>
<td>T lymphocytes</td>
<td>0/30</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>3</td>
<td>CVF</td>
<td>None</td>
<td>10</td>
<td>T lymphocytes</td>
<td>0/30</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Donors were treated with CVF at day 9 after immunization. Cells were transferred intraperitoneally. Incidence of EAAU given as positive/total eyes after clinical examination. Data are presented as the mean ± SD. Severity of inflammation on histopathologic examination was grouped as mild (1+), moderate (2+ to 3+) or severe (4+). These experiments were repeated three times (n = 5 rats/experiment) with similar results.
RESULTS

Ocular Complement Activation and EAAU

MAA injected Lewis rats were killed at different time points (n = 6/each time point): days 8, 10, 12, 14, 16, 19, 23, and 30. The presence of the C3 activation product iC3b within the eye at these time points was investigated by semiquantitative Western blot analysis and was used as the measure of ocular complement activation. The 68- and 43-kDa α-chains of iC3b were identified on Western blot in normal eyes and in the eyes of MAA-injected animals killed at different time points. The blot incubated with goat IgG isotype control antibody did not show any immunoreactivity (data not shown).

Densitometric analysis of these polypeptide chains indicated that the levels of iC3b within the eye paralleled the course of the disease. On days 8 to 10, low levels of iC3b were detected in the eyes of MAA-injected rats, similar to naive animals. Levels of iC3b increased markedly in MAA-immunized animals on days 12 to 16 after immunization and returned to baseline during the resolution of EAAU (Fig. 1A). On days 12 to 16, the intraocular levels of iC3b in MAA-immunized rats were significantly higher (P < 0.05) than other time points—specifically, days 8, 10, 19, 23, and 30 (Fig. 1A). In contrast, with a nonrelevant antigen, BSA, only low levels (similar to those in naive rats) of iC3b were detected in the eyes of BSA-injected animals at all time points (Fig. 1B). Intraocular inflammation was not observed in BSA-injected rats (Fig. 1B).

Effect of Systemic Complement Depletion on EAAU

A single injection of CVF was given at five different time points as described in the Materials and Methods section. Complement depletion 24 hours before immunization or at day 4 after immunization did not alter the course of EAAU (Table 2). Of note, the incidence, duration, and severity of disease (determined both clinically and histologically) was dramatically reduced in rats treated with CVF on day 9 after immunization compared with that in control rats (Table 2; Figs. 2A, 2B). In these animals a single injection of CVF on day 9 after immunization resulted in complete depletion of complement for 5 days (Fig. 2C). Low levels of iC3b were detected within the eye of MAA-injected, CVF-treated animals on days 16 (data not shown), 19 (Fig. 2D), 23, and 30 (data not shown). In contrast, complement depletion after the onset of inflammation (day 14 or 19) did not affect EAAU (Table 2).

Effect of Systemic Complement Depletion on Adoptive Transfer EAAU

Ten million in vitro–primed T cells isolated from the draining lymph nodes of Lewis rats killed at day 11 after immunization transferred EAAU to naive syngeneic rats (group 1, Table 3). In contrast, EAAU was completely suppressed when in vitro–primed-T cells cultured in the absence of complement were transferred to a CVF-treated, complement-depleted host (group 2, Table 3). Furthermore, T cells obtained from MAA-sensitized Lewis rats treated with CVF on day 9 after immunization and...
cultured for three days in the absence of complement did not transfer EAAU to naïve syngeneic rats (group 3, Table 3). These results suggest that complement is critical for the development of EAAU induced by either active immunizations or the transfer of in vitro–primed antigen-specific T cells.

**Effect of Systemic Complement Depletion on Intraocular Cytokine and Chemokine Production**

Lewis rats immunized with MAA were divided in two groups: One group received a single injection of CVF on day 9; the control group received PBS on the same day. Animals (n = 6/time point in each group) were killed at days 8, 10, 12, 14, 16, 19, 23, and 30 after immunization, and the eyes were harvested.

Using semiquantitative RT-PCR, we detected low levels of IFN-γ transcripts in the eyes of naïve animals, as well as on days 8 and 10 in complement-sufficient MAA immunized rats (Fig. 3A). In the presence of complement, IFN-γ transcripts within the eye increased on day 12, peaked on days 16 to 19 (Fig. 3A), returned to basal levels by day 23, and remained at this level on day 30 (data not shown). In contrast, IFN-γ mRNA remained at low levels at all time points in complement-depleted rats (Fig. 3B). IL-10 mRNA (Fig. 3A) increased modestly on day 10, reached maximum levels on days 12 to 19, and returned to basal levels by day 30 (data not shown) in complement-sufficient rats. In contrast, complement depletion resulted in decreased IL-10 mRNA levels within the eye at each time point (Fig. 3B).
In the presence of complement, low levels of IP-10 transcripts were observed on day 10 (Fig. 3C). mRNA levels of IP-10 increased on day 12, peaked on days 14 to 19 (Fig. 3C), and returned to basal levels on day 23 (data not shown). In contrast, in the absence of complement, mRNA for IP-10 was not detected at all time points studied (Fig. 3C). No bands were detected in the control experiments without RNA or reverse transcriptase (data not shown).

ELISA results (Fig. 4) demonstrated that IFN-γ protein levels increased dramatically on day 12, peaked on day 14, and returned to basal levels by day 30 in MAA-immunized, complement-sufficient rats (Fig. 4A). Complement depletion resulted in undetectable levels of IFN-γ protein on day 10, significantly reduced levels on days 12 to 19 (P < 0.05), and undetectable levels on day 30 (Fig. 4A). In complement-sufficient, MAA-immunized rats, the maximum increase of IL-10 protein was observed on days 14 to 16, with a gradual decline during the resolution of EAAU (Fig. 4B). A time course study showed that, in complement-depleted rats, the levels of IL-10 protein on days 14 to 19 were significantly lower (P < 0.05) than those in complement-sufficient animals (Fig. 4B). IL-10 protein was not detected in complement-depleted animals on day 8 but was higher than complement-sufficient rats on days 10 and 23 (P < 0.05).

Western blot analysis revealed low constitutive levels of IP-10 protein on day 10 after immunization in MAA-immunized, complement-sufficient rats (data not shown). IP-10 protein levels increased on day 12, peaked on days 14 to 19 (Fig. 4C), and were very low on days 23 to 30 in these rats (data not shown). In contrast IP-10 protein was barely detectable in complement-depleted rats at all time points (Fig. 4C). Control blots incubated with the rabbit IgG isotype control did not show any reactivity (Fig. 4D).

Expression of Cell Adhesion Molecules in Complement-Depleted Animals

We also studied the effect of complement depletion on the intraocular expression of ICAM-1 and LECAM-1 at the peak of EAAU, with immunofluorescence microscopy. MAA-immunized Lewis rats, with and without CVF treatment, were killed on day 19. Both ICAM-1 and LECAM-1 were very strongly expressed on the iris and ciliary body of complement-sufficient rats on day 19 (Figs. 5A, 5D). However, both the adhesion molecules were markedly suppressed in CVF-treated animals at this time point (Figs. 5B, 5E). Low constitutive levels of ICAM-1 (Fig. 5C) and LECAM-1 (Fig. 5F) were observed in naïve rat eye. The sections in which isotype control antibodies were substituted for the primary antibodies were negative (data not shown).

Role of iC3b and CR3 Interaction in EAAU

The eyes of MAA immunized animals killed at various time points (days 14, 16, 18, and 19) were stained with anti-rat CD11b (OX-42). This antibody is specific for the ligand binding site of CR3 (type 3 complement receptor) and inhibits the binding of iC3b to CR3.14,16 We observed the infiltration of CR3-positive cells within the iris, ciliary body, and anterior chamber on days 14 and 16 (data not shown) with massive infiltration on days 18 to 19 (Fig. 6A). No fluorescence was detected in the eyes of naïve rats (data not shown) and the sections stained with isotype control antibodies (Fig. 6B).

The in vivo effect OX-42 on EAAU was investigated and MAA-immunized rats were given intraperitoneal injections of this monoclonal antibody. We observed that anti-CR3 treat-
ment before the onset of inflammation (day 9, group 1) significantly ($P < 0.05$) reduced the severity of EAAU compared with isotype-matched, PBS-treated control subjects (Table 4). The duration of the disease was also significantly ($P < 0.05$) reduced in OX-42-treated animals compared with the control subjects (Table 4). This effect of OX-42 on EAAU was not due to the cytotoxic activity of the antibody and/or immune complexes, because the number of CR3-positive cells in the liver, kidney (data not shown), and spleen (Fig. 6) of rats after daily intraperitoneal injection of OX-42 for 6 days were similar to those injected with PBS (Figs. 6C, 6D).

DISCUSSION

Experimental autoimmune anterior uveitis (EAAU) is an autoimmune disease of the eye that closely resembles human idiopathic anterior uveitis. We used this model to explore the role of complement in the pathogenesis of ocular autoimmune disease. The data presented herein demonstrate a strong relationship between the presence–activation of complement with disease activity, as well as the expression of cytokines, chemokines, and adhesion molecules during EAAU. To the best of our knowledge this relationship has not been established previously in any type of ocular autoimmune disease.

We identified significantly increased levels of iC3b, a cleavage product of C3, within the eye during the peak of EAAU. Because activation of the complement system is necessary for the generation of C3 split products,1,17 their increased levels within the eye of MAA-immunized rats provided indisputable evidence of local complement activation during EAAU. The antigen specificity of this observation was investigated with an irrelevant antigen (BSA). The animals immunized with BSA had low levels of ocular iC3b which was similar to those in naïve rat eye. We have shown that low levels of iC3b are present in the normal rat eye.15 Taken together these results support the hypothesis that the local complement activation contributes to intraocular inflammation in EAAU.

Decomplementation of MAA-immunized Lewis rats before the onset of clinical inflammation suppressed EAAU. Of interest, complement activity in these complement-depleted rats on days 10 to 14 (i.e., during induction of EAAU) was undetectable. Complement depletion also resulted in the suppression of uveitis in adoptive transfer EAAU. These results support a central role for complement in the development of EAAU.

Complement deficiency has been reported to ameliorate various experimental autoimmune diseases.18–23 We have recently shown that activation of complement, specifically the formation of MAC, is essential for the development of laser-induced choroidal angiogenesis in mice.23

We next sought to determine how complement depletion suppressed EAAU and compared the expression of cytokines, chemokines, and adhesion molecules within the eye of both complement-sufficient and complement-depleted animals during EAAU. We studied the expression of IFN-γ, IL-10, IP-10, ICAM-1, and LECAM-1 because of their important role in the pathogenesis of various autoimmune diseases including uveitis.12,25–31 Furthermore, several studies have suggested that the expression of these cytokines, chemokines, and adhesion molecules is affected by the presence and activation of the complement system.32–41 A time course analysis of the expression of cytokines and chemokines within the eye of both complement-sufficient and -depleted rats revealed that there was a decreased production of IFN-γ, IL-10, and IP-10 in complement-depleted animals. These results imply that the release of these inflammatory mediators during EAAU was complement dependent. It has been reported by us4 and others42–44 that complement is essential for the production of cytokines such as IL-10 by antigen-presenting cells and monocytes. Analysis of cell adhesion molecule expression revealed that ICAM-1 and LECAM-1 were significantly decreased after complement depletion, indicating that these molecules are regulated by complement.

We then focused our attention on iC3b, because we noticed that the levels of iC3b were elevated in the rat eye during EAAU. The systemic injection of anti-CR3 (MRC OX-42) had a significant protective effect on EAAU, thus suggesting that the interaction of iC3b with CR3 was involved in the development of EAAU. Similar to our observations, several studies with different animal models have reported that treatment with anti-CR3 antibodies diminishes the severity of various experimental diseases.45–47 In our experiments, EAAU was not completely inhibited by anti-CR3 treatment. This may be due to the possible role of other complement activation products and receptors19–21,24,32 in EAAU.

In summary, we have demonstrated that the expression of cytokines, chemokines, and adhesion molecules necessary for the development of EAAU requires availability and activation of complement. Interference in the availability of complement by systemic depletion leads to the suppression of disease. Furthermore, the interaction between iC3b and CR3 plays an important role in EAAU. Thus, complement inhibition and/or treatment with anti-CR3 antibodies may be a novel therapy for autoimmune uveitis.

References

6. D’Ambrosio AL, Pinsky DJ, Connolly ES. The role of the comple-
ment cascade in ischemia/reperfusion injury: implications for neu-

7. Singhrao SK, Neal JW, Morgan BP, Gasque P. Increased comple-
ment biosynthesis by microglia and complement activation on


10. Broekhuyse RM, Kuhlman ED, Winkens HJ, Van Vugt AH. Exper-
mmental autoimmune anterior uveitis (EAU), a new form of ex-
perimental uveitis: I. Induction by a detergent-insoluble, intrinsic

anterior uveitis: induction with melanin-associated antigen from
the iris and ciliary body. *Invest Ophthalmol Vis Sci*. 1995;36:
1056–1066.

12. Kim MC, Kabeer NH, Tandhasetti MT, Kaplan HJ, Bora NS. Immu-
nohistochemical studies in melanin associated antigen (MAA) in-
duced experimental autoimmune anterior uveitis (EAAU). *Curr Eye

13. Bora NS, Woon MD, Tandhasetti MT, Cirrito TP, Kaplan HJ. Induc-
tion of experimental autoimmune anterior uveitis by a self-antigen:
melanin complex without adjuvant. *Invest Ophthalmol Vis Sci*. 

14. Bora NS, Sohn JH, Kang SG, et al. Type I collagen is the autoantigen
172:7086–7094.

15. Sohn JH, Kaplan HJ, Suk HJ, Bora PS, Bora NS. Chronic low level
complement activation within the eye is controlled by intraocular
41:3492–3502.

16. Robinson AP, White TM, Mason DW. Macrophage heterogeneity in
the rat as delineated by two monoclonal antibodies MRC OX-41 and
MRC OX-42, the latter recognizing complement receptor type 3.

17. Davis AE 3d, Harrison RA, Lachmann PJ. Physiologic inactivation of
complement cascade in ischemia/reperfusion injury: implications for neu-

18. Davis AE 3d, Harrison RA, Lachmann PJ. Physiologic inactivation of
complement cascade in ischemia/reperfusion injury: implications for neu-

19. Wallace GR, John Curnow S, Wloka K, Salmon M. Chronic low level
complement C9 deposition, P-selectin expression, and cellular infil-

20. Vriesendorp FJ, Flynn RE, Malone MR, Pappolla MA. Systemic
complement depletion reduces inflammation and demyelination in
adoptive transfer experimental allergic neuritis. *Acta Neuropathol
(Berl)*. 1998;95:297–301.


22. Tsunoda I, Lane TE, Blackett J, Fujinami RS. Distinct roles for IFN-
10 and CXCL10 in three animal models, Thielier’s virus infection,
EAE, and MHV infection, for multiple sclerosis: implication of dif-

23. Anderson ME, Siahaan TJ. Targeting ICAM-1/LFA-1 interaction for
controlling autoimmune diseases: designing peptide and small

52:127–141.

25. Fang IM, Yang CH, Lin CP, Yang CM, Chen MS. Expression of
chemokine and receptors in Lewis rats with experimental autoim-

26. Godau J, Heller T, Hawlisch H, et al. C5a initiates the inflamma-
tory cascade in immune complex peritonitis. *J Immunol*. 2000;173:
3437–3445.

expression in human umbilical ven endothelial cells. *Am J Pathol*. 

28. Jagels MA, Dafler PF, Hugli TE. C3a and C5a enhance granulocyte
adhesion to endothelial and epithelial cell monolayers: epithelial
and endothelial priming is required for C3a-induced costimulatory

29. Vapoorciyan AA, Mulligan MS, Warren JS, Barton PA, Miyasaka A,
Ward PA. Up-regulation of lung vascular ICAM-1 in rats is comple-

30. Monsinjon T, Gasque P, Chan P, Ischenko A, Brady JJ, Fontaine
MC. Regulation by complement C5a and C5a anaphylatoxins of
lymphocyte production in human umbilical vein endothelial cells. 

31. Jumae AE, Ischenko A, Chan P, Fontaine M. Complement com-
ponent anaphylatoxins upregulate chemokine expression by hu-

32. Ahamed J, Venkatesha RT, Thangam EB, Ali H. C3a enhances nerve
growth factor-induced NFAT activation and chemokine produc-
tion in a human mast cell line, HMC-1. *J Immunol*. 2004;172:
6961–6968.

33. Tramoni NL, Kuipers PJ, Huber CM, et al. Modulation of leuko-
cyte recruitment and IL-8 expression by the membrane attack
complex of complement (C5b-9) in a rabbit model of antigen-

34. Papagianni AA, Alecopoulos E, Leontsini M, Papadimitriou M.
C5b-9 and adhesion molecules in human idiopathic membranous

receptor in regulating the effector phase of synovial infiltration and
196:1461–1471.

231.

and down-regulation of IL-12 by iC3b deposited in ultraviolet-

drotic cell differentiation and interleukin-12 production by com-
plement iC3b via a mitogen-activated protein kinase signalling

39. Gordon EJ, Myers KJ, Dougherty JP, Rosen H, Ron Y. Both anti-
CD11a (LFA-1) and anti-CD11b (MAC-1) therapy delay the onset
and diminish the severity of experimental autoimmune encephal-

40. Paalmen MJ, Dijkstra CD, van der Ende MB, Pena AS, van Rees EP,
Anti-CD11b/CD18 antibodies reduce inflammation in acute colitis

41. Johnson LL, Gibson GW, Sayles PC. CR3-dependent resistance to
acute Toxoplasma gondii infection in mice. *Infect Immun*. 