Adhesion Molecules in Experimental Phacoanaphylactic Endophthalmitis

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Intraocular accumulation of inflammatory neutrophils is an important feature of experimental phacoanaphylactic endophthalmitis (EPE). Increasing evidence suggests that localization of neutrophils to the site of inflammation requires the participation of neutrophil and endothelial adhesion molecules. These studies were undertaken to determine if blocking of adhesion molecules on neutrophils (CD18) or endothelium (ELAM-1) could attenuate EPE in Lewis rats. Treatment of experimental animals with anti-CD18 or anti-ELAM-1 significantly suppressed intraocular neutrophil accumulation, retinal hemorrhage, and vasculitis, and attenuated retinal edema formation by 48% and 70%, respectively. These observations demonstrate that antibodies directed against adhesion molecules on the neutrophil (CD18) or the vascular endothelial cell (ELAM-1) exhibit potent anti-inflammatory effects, resulting in a striking amelioration of injury in EPE in rats. Invest Ophthalmol Vis Sci 33:3417-3423, 1992

Localization of acute inflammatory cells to the site of a noxious agent involves adhesion to the luminal surface of the vascular endothelium, movement through the endothelial junctions, detachment from the abluminal surface of the endothelial cell, and migration through the extravascular tissue. Many of the components of this process are not well understood, but recently there have been rapid developments in understanding the mechanisms of neutrophil adhesion to vascular endothelial cells.1

The identification of individuals with an absence of an adhesion molecule component (CD18) on leukocytes has created interest in the role of adhesion molecules in acute inflammation. Leukocyte adhesion deficiency disease (LAD) is characterized by recurrent infections, paucity of neutrophils in abscesses, and deficiencies in neutrophil adhesion, phagocytosis, and motility.2 LAD is caused by an absence or defect in the β chain of the heterodimer that constitutes the CD11/18 complex.3 None of the β2 integrins (LFA-1, Mac-1, p150,95) associated with cell adhesion is detected intact in cells from patients with LAD.

Antibodies to adhesion molecules on neutrophils or endothelial cells inhibit adhesion in vitro and have been demonstrated to modulate inflammation in dermal and pulmonary inflammatory reactions.4 The observations reported here were undertaken to determine if blocking of neutrophil or endothelial adhesion molecules could attenuate an acute form of uveitis—experimental phacoanaphylactic endophthalmitis (EPE).

Methods

Monoclonal Antibodies

A monoclonal antibody against rat CD18 was prepared by immunizing BALB/c mice with lymphocyte suspensions prepared from rat spleens. Hybridomas were prepared as previously described.4 One hybridoma, CL-26 (IgG1), produced antibody that exhibited binding to rat neutrophils, lymphocytes, and monocytes. Immunoprecipitation studies of 125I-surface-labeled peritoneal exudate rat neutrophils revealed a 95 kD β subunit and two α subunits consistent with CD11a and CD11b. F(ab')2 preparations were made using the ImmunoPure F(ab')2 preparation kit (Pierce Co., Rockford, IL).

Antibody directed against the endothelial leukocyte adhesion molecule-1 (ELAM-1) was generated by immunization of BALB/c mice with human umbilical vein endothelial cells (HUVEC) that had been stimulated with recombinant human IL-1β for 3 hr at 37°C. Mouse hybridomas were prepared in the usual manner, and supernatant fluids containing IgG were screened for their ability to react exclusively with stim-
ulated HUVEC, using immunoperoxidase technology. Hybridomas were subcloned and the antibodies were further characterized as previously described. The hybridoma, CL-3, a murine monoclonal antibody (IgGl) to human ELAM-1, was found to react immunohistochemically with tumor necrosis factor \( \alpha \)-stimulated rat pulmonary vascular endothelial cells. Because the CL-3 anti-ELAM-1 F(ab')\(_2\) exhibited profound protective effects in neutrophil-mediated lung injury in rats, this monoclonal antibody was also used in the present study.

**Animal Model**

Nineteen male Lewis rats (100–150 g) were sensitized to rabbit lens protein by a series of four subcutaneous injections at 2 wk intervals. The inocula were a stable emulsion consisting of, in the first injection, equal volumes of complete Freud's adjuvant (Sigma, Chemical Co., St. Louis, MO) and saline containing 10 mg rabbit lens protein. A similar emulsion of incomplete Freud's adjuvant and lens protein was used for the subsequent three injections. The lenses were injured 1 wk after the fourth sensitizing injection. The lens capsule of each animal was disrupted with a bent 30 G needle passed through the medial limbus. This produced an intraocular Arthus reaction in 100% of the animals after lens injury. Seven positive control animals were given 0.5 ml of intravenous saline injections through the dorsal penile vein at 2, 4, and 6 hr after lens injury. Six animals received intravenous injections of 33 \( \mu \)g (protein) of F(ab')\(_2\) fragments of CL-26 anti-CD18 antibody at 2, 4, and 6 hr after lens injury, and six animals received 50 \( \mu \)g of CL-3 anti-ELAM-1 F(ab')\(_2\) at the same time intervals after lens injury. The amounts of anti-ELAM-1 and anti-CD18 injected did not cause neutropenia (data not shown). Also, because control antibodies for anti-CD18 and anti-E-selectin proved uniformly negative in a series of rat studies previously published by our group, and because of the expense, injection of the same monoclonal control antibodies into additional rats was omitted. The animals were killed 24 hr after lens injury. The eyes were enucleated, fixed in 10% buffered formaldehyde solution, sectioned vertically, and processed for paraffin embedding. Tissue sections were stained with hematoxylin-eosin. All procedures conformed to the ARVO Resolution on the Use of Animals in Research.

**Histopathology**

Before histopathologic analysis, the slides were coded to make the pathologist unaware of the treatment. The principal pathologic findings consisted of retinal swelling, hemorrhage, and vasculitis; only minimal choroidal inflammation was observed. Retinal hemorrhage and vasculitis were graded 1+ to 4+, according to previously described criteria. Retinal swelling appeared suitable for morphometric analysis using a microscope eyepiece equipped with a measuring scale. A representative slide of each eye of each experimental animal was magnified (10 \( \times \) 5). The mean of five measurements taken at regular intervals was determined for each eye. The mean retinal thickness measurements of positive control and treated animals were compared using Student's t-test.

**Results**

A classical hemorrhagic vasculitis was induced by lens injury in rats that had been sensitized to rabbit lens protein. The development of vasculitis paralleled the efferent channels for outflow of lens proteins. Early vasculitis and hemorrhage were seen in the iris ciliary body and in the inner and middle vascular layers of the retina (Figs. 1A–C). There was coagulative necrosis of the vessel walls with a mixed perivascular infiltrate, predominantly neutrophils, together with extensive hemorrhage. The inflammation was observed in the episclera and sclera and in the extraocular muscles before the development of choroiditis, suggesting that lens protein reached the choroid at later stages of the inflammation. The choroidal inflammation may have been stimulated partly by retinal necrosis.

Treatment with monoclonal antibody to CD18, a neutrophil adhesion molecule, significantly suppressed the expected inflammation with a considerable reduction in neutrophil infiltration and hemorrhage (Figs. 2A and B). Antibody to the endothelial cell adhesion molecule ELAM-1 also effectively modulated the expected inflammation by interfering with neutrophil accumulation (Figs. 3A and B).

As shown in Figure 1, EPE also was accompanied by striking retinal edema. Treatment of experimental animals with anti-CD18 or anti-ELAM-1 significantly attenuated retinal edema formation, as demonstrated by morphometric analysis (Fig. 4). Compared to retinal swelling in positive control animals (302.5 \( \mu \)m), anti-CD18- and anti-ELAM-treated rats demonstrated average retinal swellings of 215.75 \( \mu \)m (\( P < 0.05 \)) and 175 \( \mu \)m (\( P < 0.001 \)) respectively. Because normal rat eyes showed a mean retinal thickness of 120 \( \mu \)m (data not shown), anti-CD18 and anti-ELAM caused a reduction in EPE inflammatory retinal swelling by 48% and 70%, respectively. As depicted in Table 1, the attenuation in retinal swelling was accompanied by comparable reductions in retinal hemorrhage and vasculitis.
These observations demonstrate that monoclonal antibodies directed against adhesion molecules on the inflammatory neutrophil (CD18) or the vascular endothelial cell (ELAM-1) exhibit potent anti-inflammatory effects, resulting in a striking amelioration of experimental phacoanaphylactic endophthalmitis in the rat.

Discussion

Increasing evidence suggests that the development of Arthus-type, acute EPE largely is mediated by oxygen-derived free radicals generated by intraocularly accumulating inflammatory neutrophils. The importance of neutrophils in the pathogenesis of EPE is further emphasized by our present observation that antibody to the neutrophil β2 integrins (CD11/18) and antibody to the vascular endothelial cell adhesion molecule, ELAM-1, markedly attenuated intraocular inflammation, demonstrating a critical role for these molecules in the accumulation of neutrophils and the resulting injury. These observations are supported by recent findings by Mulligan et al., who demonstrated an obligate role for ELAM-1 in the recruitment of neutrophils in experimental IgG immune complex-induced vasculitis. The authors showed that anti-ELAM-1 markedly reduced vascular injury after IgG immune complex deposition in dermis and lungs of rats. As to be expected, the protective effects of anti-ELAM-1 were related to greatly diminished recruitment of neutrophils. Immunohistochemical analyses revealed a significant up-regulation of ELAM-1 in the lung vasculature, reaching peak intensities 3–4 hr after the deposition of immune complexes in the lungs.

Several neutrophil and endothelial cell adhesion molecules are involved in neutrophil adhesion to vascular endothelium (Fig. 5). In addition to adhesion, some of these molecules are involved in diapedesis or migration across the endothelium. The endothelial adhesion receptors ELAM-1 and GMP-140, as well as the leukocyte adhesion molecule LECAM-1, represent a family of proteins that are named E-, P-, and L-selectin, respectively, to reflect the involvement of carbohydrate recognition in their functions.
nizing sialylated forms of the Lewis x glycan on resting or activated neutrophils.16–18

The platelet granular membrane protein-140 (GMP-140, P-selectin) is present in the alpha granules of platelets and in the Weibel-Palade bodies of endothelial cells.19,20 Upon stimulation with histamine or

The endothelial leukocyte adhesion molecule-1 (ELAM-1, E-selectin), another member of the selectin family, has been shown to mediate neutrophil adhesion to tumor necrosis factor or IL-1-activated endothelial cells,14 which express ELAM-1 on their surface within 2–4 hr after cytokine treatment.15 As GMP-140, ELAM-1 is known to function as a lectin, recog-

Fig. 2. Experimental phacoanaphylactic endophthalmitis with anti-CD18 treatment. (A) The anterior segment shows minimal inflammation at the iris and site of lens capsule disruption. The peripheral retina is uninvolved. (Hematoxylin-eosin, original magnification X412.) (B) The posterior segment is free of inflammation. (H-E, original magnification X412.)

Fig. 3. Experimental phacoanaphylactic endophthalmitis treated with antibody against ELAM-1. (A) Anterior segment with minimal acute inflammation at the site of lens capsule disruption. There is mild iritis and minimal vasculitis in the peripheral retina. (Hematoxylin-eosin, original magnification X412.) (B) The posterior segment is free of inflammatory reactions (H-E, original magnification X412.)
thrombin, GMP-140 is rapidly (within minutes) moved to the endothelial cell surface, where it mediates adhesion of neutrophils21,22 and thus promotes rapid accumulation of these cells at sites of acute inflammation. Recent observations suggest that binding of leukocytes to GMP-140 is mediated by the carbohydrate ligand sialyl-Lewis x.23

The neutrophil L-selectin, lectin adhesion molecule-1 (LECAM-1), which has been shown to contain sialyl Leα24 is expressed on unstimulated neutrophils and appears to be involved in the initial adhesion of neutrophils to cytokine-stimulated vascular endothelial cells.25-29 For example, von Andrian et al, with the use of intravital video microscopy, demonstrated that anti-LECAM-1 monoclonal antibody inhibits the initial reversible neutrophil rolling along the vascular endothelium in rabbit mesenteric venules.30 Upon chemotactic activation of the neutrophil, LECAM-1 is rapidly shed from the cell surface and does not seem to play a role in the transendothelial migration of neutrophils.26-29 The endothelial lectin ligand for LECAM-1 may well be ELAM-1 or GMP-140.24

The same inflammatory cytokines (tumor necrosis factor, IL-1) that induce synthesis and expression of ELAM-1 also induce intercellular adhesion molecule-1 (ICAM-1).31,32 However, the endothelial expression of ICAM-1 is somewhat delayed, reaching peak values within 24 hr after tumor necrosis factor or IL-1 stimulation in vitro.33 The ICAMs are members of the immunoglobulin gene super family32-34 and bind to lymphocyte function-associated antigen-1 (LFA-1), a β2 integrin (CD11α/CD18) that is constitutively expressed on all leukocytes. ICAM-1 also can serve as a ligand for the β2 integrin, Mac-1 (CD11b/CD18), which is expressed on activated neutrophils and monocytes.36 The β2 integrin, P150,95 (CD11c/CD18), is expressed on neutrophils, monocytes, macrophages, some T and B lymphocytes, and on hairy cell leukemia cells. P150,95 has considerable homology to Mac-1. Recent evidence indicates that P150,95 has an independent endothelial binding capacity, but is much less avid than LFA-1.37

Of the three β2 integrins,1 LFA-1 (CD11a/CD18) and Mac-1 (CD11b/CD18) are known to play an important role in the adhesion of neutrophils to vascular endothelium. Furthermore, the interaction between the β2 integrins and ICAM-1 appears to be essential for the transendothelial migration or extravasation of neutrophils. Monoclonal antibodies to CD18 can almost completely block the migration of chemotactically activated neutrophils across monolayers of cytokine-stimulated endothelial cells in vitro.5,38 These in vitro data are supported by corresponding in vivo observations. Our present studies in rats demonstrate that antibody to CD18 significantly reduces ocular influx of neutrophils and thus attenuates the development of EPE. Others have shown that systemic treat-

![Fig. 4. The effects of antibodies to adhesion molecules on experimental phacoanaphylactic endophthalmitis.](image)

![Fig. 5. A model of associated neutrophil and endothelial cell adhesion molecules.](image)

<table>
<thead>
<tr>
<th>Condition</th>
<th>n*</th>
<th>Hemorrhage†</th>
<th>Vasculitis‡</th>
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<tr>
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* Number of animals.
† Retinal hemorrhage and vasculitis were graded 1+ to 4+ according to previously described criteria and evaluated at 24 hr after mechanical rupture of the eye lens capsule in lens protein-sensitized rats.
‡ Experimental animals received intravenous injections of 33 μg and 50 μg F(ab')2 fragments of monoclonal antibody against CD18 and ELAM-1, respectively. The antibody preparations were administered at 2, 4, and 6 hr after lens injury.

![Table 1. Effect of anti-CD18 and anti-ELAM-1 on retinal hemorrhage and vasculitis in experimental phacoanaphylactic endophthalmitis in rats.](image)
ment of experimental animals with antibody against CD11, CD18, or monoclonal antibody to ICAM-1 reduces emigration of neutrophils in response to various experimental inflammatory stimuli. Our observation that antibody against ELAM-1 also provides significant protection from EPE may be explained by the presumption that selectins, although primarily involved in neutrophil margination, provide the means for the subsequent transendothelial migration that involves primarily the CD18 integrins and ICAM-1.

In summary, anti-CD18 and anti-ELAM-1 antibodies markedly inhibited the Arthus-type ocular inflammatory response in rats. This demonstrates the importance of adhesion molecules on neutrophils (CD11/CD18 complex) and endothelial cells (ELAM-1) for the ocular accumulation of inflammatory neutrophils that is essential for the full expression of experimental phacoanaphylactic endophthalmitis.

**Key words:** adhesion molecules, CD18, ELAM-1, phacoanaphylactic endophthalmitis, uveitis

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**References**


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