Endothelial Leukocyte Adhesion Molecule-1 in Endotoxin-Induced Uveitis

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Expression of endothelial leukocyte adhesion molecule-1 (ELAM-1) on endothelial cells leads to the attachment of polymorphonuclear leukocytes. The sequential expression of ELAM-1 and major histocompatibility complex (MHC) class II antigen was examined in the eyes of 59 Lewis rats with endotoxin-induced uveitis (EIU) after the injection of Salmonella typhimurium endotoxin. The eyes were enucleated at 2-hr intervals. Hematoxylin and eosin-stained paraffin-embedded sections and immunohistochemically stained cryostat sections were graded by two masked observers. The MHC class II antigen was expressed on cells in the iris and ciliary body 4 hr after injection of endotoxin and on the corneal endothelium, 8 hr postinjection. It was found that ELAM-1 was expressed first on cells of the ciliary body and iris 10 hr after the injection of endotoxin and on the corneal endothelium, 22 hr postinjection. Clinical and histopathologic disease developed 16 hr postinjection. Adherence of polymorphonuclear cells to the corneal endothelium was observed at the time of ELAM-1 expression. In conclusion, expression of ELAM-1 on ocular tissue occurred in EIU and appeared to promote polymorphonuclear cell accumulation in the anterior segment of the eye. Invest Ophthalmol Vis Sci 33:2626–2630, 1992

Local or systemic injection of the lipopolysaccharide (LPS) constituents of the cell wall of gram-negative bacteria will produce inflammation in several organs, including the eye.1 Injection of LPS into the footpad of certain animals, including species of rats, mice, and rabbits, produces an anterior uveitis (characterized by iris hyperemia) and increased protein and inflammatory cell accumulation in the anterior uvea and anterior chamber. This inflammatory process is termed endotoxin-induced uveitis (EIU), and it is used as an animal model for ocular inflammation. It is associated with an increase in systemic vascular permeability2 and upregulation of major histocompatibility complex (MHC) class II antigen expression on ocular tissue.3

Studies suggest that inflammation causes endothelial cells to express cell-adhesion molecules.4 Expression of some of these molecules is important for both leukocyte homing and migration through vascular endothelium into inflammatory sites.5,6 Cell-adhesion molecules are divided into three structural groups: the immunoglobulin gene superfamily, the integrin family, and the selectins. Intercellular adhesion molecule-1 (ICAM-1) and vascular adhesion molecule-1 are two such molecules of the immunoglobulin gene superfamily that are expressed on endothelium during inflammation and bind leukocytes.4 A third cell-adhesion molecule, endothelial leukocyte adhesion molecule-1 (ELAM-1), mediates attachment of neutrophils to endothelial cells in vitro and appears to be important in the recruitment of neutrophils in a local endotoxin response in the skin.7 Because migration and accumulation of neutrophils in the anterior segment of the eye is an integral part of the inflammatory response in EIU, we studied the sequential expression of ELAM-1 and in rats with EIU and compared its expression with the expression of class II antigen.

Materials and Methods

We induced EIU in 59 female Lewis rats weighing 200 g (Charles River, Wilmington, MA) in three separate experiments by injecting 100 μg of Salmonella typhimurium endotoxin (LPS; Difco, Detroit, MI) into one footpad. All animals were treated in accordance with ARVO Resolution on Use of Animals in Research. Their eyes were examined for clinical signs of inflammation under an operating microscope. The
Table 1. Kinetics of MHC class II antigen and ELAM-1 expression in EIU

<table>
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<tr>
<th>Time after injection of endotoxin (hr)</th>
<th>Findings</th>
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<td>4</td>
<td>Class II antigen expressed on cells in the iris and ciliary body</td>
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<tr>
<td>8</td>
<td>Class II antigen expressed on the corneal endothelium</td>
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<td>10</td>
<td>ELAM-1 expressed on the vascular endothelium and on resident cells in the iris and ciliary body</td>
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<td>Clinical and histopathologic evidence of disease</td>
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MHC, major histocompatibility complex.

animals were killed, and both eyes were enucleated at 2-hr intervals from the time of injection until 48 hr postinjection. One eye was placed in 10% buffered formalin for routine histopathologic examination. The other eye was embedded in OCT (Miles, Naperville, IL) and snap frozen.

Formaldehyde-fixed eyes were embedded in methylmethacrylate, and 3-μm thick sections were stained with hematoxylin and eosin. Immunohistochemical staining was done on 5-μm frozen sections using an avidin–biotin–peroxidase complex method. The primary antibodies included a monoclonal antibody against the rat MHC class II antigen RT1B (OX6; Sera-lab, Westbury, NY) at a concentration of 1:100 and a monoclonal antibody against ELAM-1 (anti-human ELAM-1 that cross reacts with rat ELAM-1; courtesy of Dr. M. P. Bevilacqua, Harvard Medical School) at a concentration of 1:500. Mouse ascites fluid containing 1–2 μg/ml of protein was the control primary antibody, and biotin-conjugated goat anti-mouse immunoglobulin G was the secondary antibody. The slides were graded by two masked observers. The degree of immunohistochemical staining was compared with a normal eye and graded according to a scale previously published.3

Results

Clinical and histopathologic evidence of inflammation was found 16 hr postinjection. The clinical signs included iris hyperemia and a fibrinous exudate in the anterior chamber. On pathologic examination, neutrophils and monocytes were seen in the iris and ciliary body. Proteinaceous material, fibrin debris, and neutrophils were evident in the anterior chamber, with many neutrophils adherent to the corneal endothelium.

The time course for the expression of MHC class II antigen and ELAM-1 in the anterior segment of the eye after induction of EIU are shown in Table 1. At the time of endotoxin injection, MHC class II antigen was expressed weakly on one or two resident cells in the stroma of the ciliary body and iris. By 4 hr postinjection, MHC class II antigen was expressed on epithelial and stromal cells of the iris and ciliary body. By 8 hr postinjection, MHC class II antigen was expressed on the corneal endothelium and scattered keratocytes. We found MHC class II antigen persisted on ocular tissue 48 hr after injection.

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Fig. 1. Light micrograph of the cornea 22 hr after injection of Salmonella typhimurium. Immunohistochemical staining with a monoclonal antibody against RT1B (rat [MHC] class II antigen) shows expression of MHC class II antigen on the corneal endothelium and some keratocytes (arrows). (×400.)
The ELAM-1 did not appear to be expressed constitutively on any ocular tissue, but it was expressed first on stromal and vascular endothelial cells in the iris and ciliary body 10 hr after injection of LPS (Fig. 2A). Later, ELAM-1 was expressed on the corneal endothelium (22 hr postinjection, Fig. 3A). At this time, neutrophils were present in the anterior chamber, and many were adherent to the corneal endothelium where ELAM-1 was expressed. At 24-48 hr after injection, expression of ELAM-1 on the corneal endothelium and cells in the ciliary body and iris gradually diminished. By contrast, ocular tissue did not stain with the control antibody (Figs. 2B, 3B).

Discussion

These results suggest that ELAM-1 is an inducible cell adhesion molecule, which was expressed on vascular endothelial cells in the iris and ciliary body at the onset of EIU. It was expressed on the corneal endothelium at the peak of inflammation. A cell surface glycoprotein, ELAM-1 is expressed by cytokine-activated endothelium that predominantly binds neutrophils. Others showed that skin-homing memory T-lymphocytes bound to ELAM-1 transfected COS cells, and ELAM-1 contributed to the greater adhesion to endothelium of memory T cells than naive T cells. In one study, the role of ELAM-1 on the recruitment of neutrophils in the local endotoxin response in the skin of baboons was examined. Endothelium in control skin did not express ELAM-1, but this developed 2 hr after injection of endotoxin with concurrent extensive adhesion and extravasation of neutrophils. Subsequently, ELAM-1 expression decreased; it disappeared by 9 hr.

In our experiment, ELAM-1 was expressed first in the eye 8 hr after injection of LPS. It gradually diminished 24-48 hr after injection. However, we injected endotoxin into the footpad of rats; therefore, the resultant ocular inflammation was not a local endotoxin response. This probably explains the delay in ELAM-1 expression in the eye. Such expression on the vascular endothelium of the ciliary body and iris occurred before neutrophils could be identified histopathologically, suggesting a role in neutrophil recruitment. Neutrophils adhered to the corneal endothelium after ELAM-1 expression. Corneal and vascular endothelium are derived from different embryonic tissues. Corneal endothelium develops from cranial neural crest cells, and the endothelium of ocular blood vessels is derived from mesoderm. Nevertheless, when the the expression of ICAM-1 was studied on the corneal endothelium and corneal stroma keratocytes, it was found that antibody to ICAM-1 effectively blocked neutrophil binding to corneal endothelium of cell cultures and whole corneas. The exact role of ELAM-1 in neutrophil binding to the corneal endothelium cannot be determined from this study, and investigation of the effect of anti-ELAM-1 antibody on the development of endotoxin-induced uveitis is warranted.

The accumulation of neutrophils in the anterior chamber of the eye and the adherence of leukocytes to the corneal endothelium are important clinical signs of ocular inflammation. Previous studies show that ICAM-1 is important in neutrophil binding to the corneal endothelium. Ours is the first study (to our knowledge) to demonstrate the expression of ELAM-1 on ocular tissue. This substance was not expressed...
constitutively but appeared on vascular endothelium of the iris and ciliary body before clinical and histopathologic evidence of inflammation and on the corneal endothelium 22 hr after the induction of EIU. This study suggests that the expression of ELAM-1 may play a role in neutrophil binding during the early phases of ocular inflammation and that anti-ELAM-1 antibody may be useful therapeutically to block neutrophil recruitment and adhesion to ocular tissues.

Key words: ELAM-1, cell adhesion molecules, endotoxin-induced uveitis, ocular inflammation, endothelium

References
5. Bevilacqua MP, Stengelin S, Gimbrone MA Jr, and Seed B.


