Recurrent Intraocular Inflammation in Endotoxin-Induced Uveitis

Alexander T. Kozbich, Chi-Chao Chan, Igal Gery, and Scott M. Whitcup

PURPOSE. Endotoxin-induced uveitis (EIU) in rats and mice peaks 24 hours after endotoxin injection and is commonly assumed to be a monophasic disease. This study examined intraocular inflammation at later time points to determine whether endotoxin injection can induce recurrent intraocular inflammation in strains of mice with high or moderate levels of susceptibility to EIU.

METHODS. EIU was elicited in two mouse strains with high (C3H/HeN) and moderate (FVB/N) susceptibility, by means of intraperitoneal injections of Salmonella typhimurium endotoxin. Inflammatory cells in the anterior and posterior segments of the eye were counted by a masked observer on histologic sections of eyes from 1 to 17 days after endotoxin injection.

RESULTS. A bimodal distribution of inflammatory cell infiltration was noted in eyes from C3H/HeN mice. As previously reported, inflammation peaked at 24 hours after endotoxin injection. However, a second, more pronounced peak of intraocular inflammation occurred approximately 5 days after endotoxin injection. FVB/N mice had a single peak of intraocular inflammation 4 days after injection.

CONCLUSIONS. Endotoxin injection in C3H/HeN elicits recurrent intraocular inflammation. The previously unrecognized second peak of inflammation is more severe than the initial inflammatory disease. Studies on this second inflammatory peak may be useful in determining the pathogenesis of recurrent uveitis in humans. (Invest Ophthalmol Vis Sci. 2000;41:1823–1826)

Endotoxin, when injected at sites far from the eye, elicits acute uveitis. This endotoxin-induced uveitis (EIU) is a useful animal model for acute uveitis in humans. Ayo first demonstrated that a single intravenous injection of endotoxin induces ocular inflammation. EIU was easily elicited in dogs, cats, and rabbits; however, smaller laboratory animals were resistant to the disease. Rosenbaum et al. induced EIU in Lewis rats with intravenous, intraperitoneal, or intrafootpad injections of endotoxin, and Forrester et al. showed EIU induced by intracocular injection of endotoxin in Columbia–Sherman rats. EIU has also been described in C3H/HeN mice, allowing the study of acute ocular inflammation in inbred mice.

EIU is characterized in the rat predominantly by signs of inflammation in the anterior segment of the eye including iris hyperemia, miosis, increased aqueous humor protein, and inflammatory cell infiltration into the anterior uvea and anterior chamber. However, in mice the prevalent feature is inflammatory cell infiltration into the vitreous in the area of the optic nerve head. Inflammation is thought to result from mediators released by activated cells including macrophages. Release of oxygen metabolites and leukotriene B4 from endotoxin-primed neutrophils and the production of cytokines including tumor necrosis factor (TNF), interleukin (IL)-1, IL-6, and IL-8 are also thought to be important in the pathogenesis of the disease.

Finally, upregulation of cell adhesion molecules on the vascular endothelium is critical to the homing and migration of leukocytes into the eye.

When EIU is elicited with a footpad or intraperitoneal injection of endotoxin, inflammatory cells first migrate into the eye approximately 6 hours after endotoxin injection. Studies have shown that the uveitis peaks approximately 24 hours after endotoxin injection and subsides over the next 48 hours. Recurrent ocular inflammatory disease has not been described in EIU; however, uveitis has not been systematically assessed in animals more than 1 week after endotoxin injection. In this study, we investigated the histologic signs of inflammation in two strains of mice from 1 to 17 days after endotoxin injection. We show for the first time that ocular inflammation occurs in a bimodal distribution in C3H/HeN mice without additional administration of endotoxin or other immunologic stimuli.

METHODS

Animals

Female C3H/HeN and FVB/N mice, 6 to 8 weeks of age, were obtained from the National Cancer Institute’s breeding facility (Frederick, MD). The mice were kept under standard pathogen-free conditions and used at 9 to 10 weeks of age. The research adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Induction of EIU

EIU was elicited in C3H/HeN mice with an intraperitoneal injection containing 50 μg of Salmonella typhimurium endo-
toxin (Lot 51974JA, median lethal dose = 11.06 mg/kg; Difco, Detroit, MI) diluted in 0.1 ml sterile saline. EIU was elicited in FVB/N mice, known to be less susceptible to disease, with an intraperitoneal injection containing 75 μg endotoxin diluted in 0.1 ml sterile saline.

**Assessment of Disease**

C3H/HeN mice were euthanatized daily for the first 10 days and then 14 and 17 days after endotoxin injection. FVB/N mice were euthanatized daily for 6 days after injection.

Both eyes were enucleated and placed in 4% glutaraldehyde for 30 minutes and transferred into 10% buffered formalin for routine histopathology. Hematoxylin and eosin-stained 4-μm methylmethacrylate-embedded, anterior-posterior vertical sections containing the cornea, pupil, and optic nerve were prepared. The number of inflammatory cells in the anterior and posterior segments of the eye were then counted by a masked observer. The number of inflammatory cells for each animal was the mean value of both eyes. In addition the ratio of neutrophils to mononuclear leukocytes in the infiltrating inflammatory cells was also assessed by a masked observer.

**RESULTS**

Figure 1 shows the mean number of infiltrating inflammatory cells in the eyes of C3H/HeN mice at different time points after endotoxin injection. A bimodal distribution of inflammatory cells over time was noted. Similar to previous studies, there was no intraocular inflammation before endotoxin injection. Ocular inflammation peaked 24 hours after endotoxin injection and then subsided. Of interest, a second peak of intraocular inflammation, greater than the initial inflammation, was noted 5 to 6 days after endotoxin injection. These results were confirmed in a second experiment (not shown). The two peaks differed markedly in the distribution of inflammatory cells in the anterior and posterior segments of the eye. As seen in Figure 1, the majority of cells of the first peak accumulated in the posterior segment, whereas cells of the second peak localized mainly in the anterior segment. As expected, neutrophils predominated in the first peak of ocular inflammation (Fig. 2A); however, during the second peak of inflammation, mononuclear leukocytes, including lymphocytes and macrophages, predominated (Fig. 2B). The ratio of infiltrating neutrophils to mononuclear leukocytes was 3:2 (1 day after endotoxin injection), 1:2 (3 days after endotoxin injection), 1:3 (5 days after endotoxin injection), and 1:2 (7 days after endotoxin injection). A different pattern of inflammation was noted in the more disease-resistant FVB/N mice. These animals developed less severe ocular inflammation than C3H/HeN mice and did not have a bimodal distribution of intraocular inflammation. The greatest numbers of inflammatory cells in the FVB/N mice, however, were noted 4 days after injection (Fig. 3). In these mice, neutrophils were the predominant inflammatory cell infiltrating the eye throughout the disease.

**DISCUSSION**

Our data show that endotoxin-susceptible C3H/HeN mice have a bimodal pattern of ocular inflammation after a single injection of bacterial endotoxin. Previous studies report a single peak of intraocular inflammation approximately 24 hours after endotoxin injection. This inflammation decreases over the next 48 hours. However, our study showed a second, more prominent peak of intraocular inflammation occurring in the sensitive C3H/HeN mice 5 to 6 days after endotoxin injection and then rapidly subsiding over the next 4 days. A small number of remaining inflammatory cells could be found in eyes 14 days after endotoxin injection. The more endotoxin-resistant FVB/N mice did not have a bimodal pattern of ocular inflammatory disease. However, similar to the C3H/HeN mice,
intraocular inflammation first occurred 24 hours after endotoxin injection and peaked 4 days after injection.

It is noteworthy that no second peak of inflammation was recorded by Kosigo et al., who were the first to describe EIU in C3H/HeN mice. This difference between the two studies could be attributed to variability in the endotoxin batch, the injected dose, and animal source and husbandry. In addition, Kosigo et al. did not examine mouse eyes between 3 and 7 days after injection and it is conceivable that a second peak developed and subsided during the 4-day interval.

The exact mechanism for the late phase of inflammation in this model has not been fully elucidated. However, differential production of cytokines may explain the bimodal distribution of inflammation. A number of studies suggest that IL-6 may be responsible for the initial inflammatory disease. More recent data suggest that later production of IL-1 and TNF-α may explain the second peak of inflammatory disease, although in rat EIU, these cytokines are involved in the early inflammatory process.

EIU is an important model for understanding disease pathogenesis and testing new therapies for ocular inflamma-

**FIGURE 2.** Histopathologic sections of the anterior chamber of the eye. Neutrophils predominantly infiltrated the eye 1 day after endotoxin injection (A). In contrast, macrophages were the predominant infiltrating cell 5 days after endotoxin injection (B). Hematoxylin and eosin; original magnification, ×400.
tory disease. Further studies on the late phase of inflammation in this animal model may provide insight into the pathogenesis of anterior uveitis in humans, a disease that is remitting and relapsing. The model will also allow investigators to study the effect of therapy on the relapsing form of the disease, which more closely mimics human disease.

**References**