The Role of Mast Cells in Endotoxin-Induced Uveitis
Qian Li, Scott M. Whitcup, Yujiro Fujino, Robert B. Nussenblatt, and Chi-Chao Chan

Purpose. To investigate the role of mast cells in the induction of endotoxin-induced uveitis (EIU).

Methods. We previously showed that the mean mast cell number in the anterior uvea was greatest for Lewis rats, lowest for Brown Norway (BN) rats, and intermediate for LBNF1 rats. In the current experiment, we assessed the time of onset and severity of EIU in these three rat strains. We then studied changes in mast cell numbers in the anterior uvea during the induction period of EIU.

Results. Time of onset and severity of EIU were related to the number of mast cells in the anterior uvea. EIU occurred earliest in the Lewis rats, and the maximum mean grade of ocular inflammation on a scale of 0 (no inflammation) to 4 (severe inflammation) ± standard error of the mean was 3.87 ± 0.13 for Lewis rats, 1.06 ± 0.06 for BN rats, and 1.19 ± 0.12 for LBNF1 rats. The difference between the mean grade of inflammation in the Lewis rats and the other two strains was highly statistically significant (P < .001). In the Lewis rats, mast cell numbers ± SEM decreased from 68.9 ± 10.8 at baseline to 49.6 ± 5.9 4 hr after lipopolysaccharide (LPS) injection and to 27.6 ± 8.4 8 hr after LPS injection, suggesting that mast cell degranulation occurs before the development of EIU.


Injection of lipopolysaccharide (LPS) at sites far from the eye can induce a bilateral ocular inflammatory disease in susceptible animals, such as rats.1 The resulting ocular inflammation is called endotoxin-induced uveitis (EIU). However, the exact mechanism of uveitogenesis of LPS is not fully understood. EIU is characterized by the acute infiltration of neutrophils and macrophages into the anterior chamber of the eye, with protein accumulation in the aqueous humor and vitreous in severe case.1 Increased levels of thromboxane B2, prostaglandin E2, leukotriene B4, and substance P are found in eyes with EIU.1,2 Many of these inflammatory mediators are released by mast cells,3 suggesting that mast cell degranulation may contribute to the inflammatory process of EIU. However, the role of mast cells in EIU has not been well studied.

Previous studies have revealed that the susceptibility to experimental autoimmune uveitis (EAU) and experimental autoimmune encephalomyelitis (EAE) are different among different rat strains.4,5 This difference in disease susceptibility was not attributable to variation in the inducibility of major histocompatibility class II antigen,5 but more likely related to variability in the release of inflammatory mediators.6 Because the severity of posterior uveitis was related to mast cell numbers in EAU,4 we hypothesized that the susceptibility to anterior uveitis in EIU may be related to mast cell numbers in the anterior uvea (iris and ciliary body).

MATERIALS AND METHODS. Inbred female Lewis rats, Brown Norway (BN) rats, and Lewis/BN F1 hybrids (LBNF1) were obtained from Harlan Sprague Dawley (Frederick, MD) and Charles River Raleigh (Raleigh, NC). All rats were 6–8 wk of age, weighed 180–200 g, and were treated according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

EIU was induced by injecting 0.1 mg of Salmonella typhimurium endotoxin (DIFCO, Detroit, MI) suspended in 0.1 ml of sterile physiologic saline solution into one hind footpad.

Lewis, BN, and LBNF1 rats were killed by CO2 inhalation 8, 12, 16, 20, 24, 32, 40, 48, and 72 hr after endotoxin injection. Both eyes were enucleated and placed in 4% glutaraldehyde solution for 20 min and transferred to 10% buffered formalin for at least 24 hr before processing for histology. Eyes were embedded in methacrylate, and 4–6 μm vertical sections through pupillary-optic nerve head plane were stained with hematoxylin-eosin. The severity of ocular inflammation then was graded by two independent, masked observers, according to the following scale based on the number of cells infiltrating into the anterior chamber:
TABLE 1. Induction of EIU in Various Strains of Rats

<table>
<thead>
<tr>
<th>Strain</th>
<th>8</th>
<th>12</th>
<th>16</th>
<th>20</th>
<th>24</th>
<th>32</th>
<th>40</th>
<th>48</th>
<th>72</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lewis</td>
<td>0/8*</td>
<td>8/8</td>
<td>8/8</td>
<td>8/8</td>
<td>8/8</td>
<td>8/8</td>
<td>8/8</td>
<td>8/8</td>
<td>8/8</td>
</tr>
<tr>
<td>BN</td>
<td>0/8</td>
<td>4/8</td>
<td>5/8</td>
<td>8/8</td>
<td>7/8</td>
<td>7/8</td>
<td>8/8</td>
<td>8/8</td>
<td>8/8</td>
</tr>
<tr>
<td>LBNF1</td>
<td>0/8</td>
<td>5/8</td>
<td>3/8</td>
<td>5/8</td>
<td>8/8</td>
<td>8/8</td>
<td>8/8</td>
<td>8/8</td>
<td>8/8</td>
</tr>
</tbody>
</table>

* Number of positive eyes with EIU out of number of examined eyes.

EIU was induced in Lewis, BN, and LBNF1 rats as described above. Rats were killed by CO2 inhalation 0, 4, and 8 hr after LPS injection, and both eyes were immediately enucleated and placed in 10% buffered formalin for 15 min. Under a dissecting microscope, the globe was opened by an equatorial incision at the limbus, and the iris and ciliary body were carefully removed with a dissecting needle. The anterior uveal tissue then was floated in distilled water and flatly mounted on poly-1-lysine slides. The tissue was air-dried and refrigerated at 4°C for a minimum of 48 hr before staining.

The flat-mounted tissue was depigmented in aqueous potassium permanganate solution for 60 min. A 1% concentration was used for the BN and LBNF1 rats, and a 0.25% concentration was used for the Lewis rats, which have no uveal pigmentation. All slides were washed three times in distilled water and bleached with 5% aqueous oxalic acid solution for approximately 10 min until the tissue became clear. The slides again were washed in distilled water, and 0.15% toluidine blue solution was applied for 3 min. The slides were covered with Gelvatol 20/30 resin (Indian Orchard Plant, Springfield, MA) and stored at 4°C. Total number of mast cells in the iris and ciliary body of each eye was counted and recorded by two masked observers. The numbers of mast cells for both eyes were averaged for each animal.

RESULTS. The time of onset of intraocular inflammation after endotoxin injection for all three strains of rats is listed in Table 1. EIU is characterized with bilateral symmetrical acute anterior inflammation. No rats had ocular inflammation 8 hr after endotoxin injection. However, by 12 hr after endotoxin injection, all eight Lewis rats developed EIU. In contrast, only four of eight BN rats and five of eight LBNF1 rats had evidence of EIU 12 hr after endotoxin injection. Lewis rats not only developed ocular inflammation earlier, but also had more severe disease than the BN or the LBNF1 rats at all time points after endotoxin injection. Lewis rats not only developed ocular inflammation earlier, but also had more severe disease than the BN or the LBNF1 rats at all time points after endotoxin injection. Lewis rats not only developed ocular inflammation earlier, but also had more severe disease than the BN or the LBNF1 rats at all time points after endotoxin injection.
endotoxin injection (Fig. 1). The mean histologic grades of inflammation for each rat strain ± SEM are illustrated in Figure 2. Peak inflammation for all three rat strains occurred 40–48 hr after endotoxin injection. The maximum histologic grade of EIU (mean ± SEM) was 3.87 ± 0.13 for Lewis rats, 1.06 ± 0.06 for BN rats, and 1.19 ± 0.12 for LBNF1 rats. The difference in maximum inflammation between the Lewis rats and either the BN or LBNF1 rats was statistically significant (P < 0.001) using Wilcoxon’s signed rank test for unpaired observations. The difference in the maximum grade of EIU between the BN and LBNF1 rats was not statistically different (P = 1).

The mean number of mast cells in the anterior uvea for all three rat strains at baseline and during the induction period for EIU are listed in Table 2. The differences in mast cell numbers between the three strains all were statistically significant (P < 0.001 or P < 0.004) using Wilcoxon’s signed rank test for unpaired observations. Four hours after endotoxin injection, the early induction period for EIU, there was a mild decrease in mast cell number in the anterior uvea for the Lewis rats (P = 0.07). There was no significant change in mast cell number 4 hr after injection for the BN rats and the LBNF1 rats (P = 0.34 and P = 0.36). Eight hours after endotoxin injection, there was a further decrease in the number of anterior uveal tract mast cells in the Lewis rats (P = 0.02), no significant change in mast cell number for the LBNF1 rats (P = 0.09), and a small but significant increase in mast cell number in the BN rats (P = 0.002).

**DISCUSSION.** Our current data show that susceptibility to EIU is correlated with the number of mast cells in the anterior uveal tract. Lewis rats, with significantly more mast cells in the iris and ciliary body, developed ocular inflammation earlier and with greater severity than the BN and LBNF1 rats. In addi-

**TABLE 2. Numbers of Mast Cells in Iris and Ciliary Bodies in Different Strains of Rats Before and After LPS Injection**

<table>
<thead>
<tr>
<th>Strains</th>
<th>Baseline</th>
<th>4 hr After LPS Injection</th>
<th>8 hr After LPS Injection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lewis</td>
<td>68.9 ± 10.8 (15)</td>
<td>49.6 ± 5.9 (12)</td>
<td>27.6 ± 8.4 (8)</td>
</tr>
<tr>
<td>LBNF1</td>
<td>4.6 ± 1.9 (9)</td>
<td>4.6 ± 1.9 (10)</td>
<td>3.8 ± 1.4 (8)</td>
</tr>
<tr>
<td>BN</td>
<td>0.3 ± 0.6 (13)</td>
<td>1.3 ± 0.7 (9)</td>
<td>2.8 ± 0.8 (8)</td>
</tr>
</tbody>
</table>

Figures are mean ± SEM, with number of examined rats in parentheses.
tion, the maximum grade of ocular inflammation was over 3.5-fold greater in the Lewis rats than in the other two rat strains. BN rats, with almost no mast cells in the anterior uvea, were much more resistant to developing EIU.

Mast cells can be activated by a number of mechanisms, including Fce receptor cross-linkage, pharmacologic compounds, and anaphylatoxins. Mast cell activation causes degranulation and the release of preformed inflammatory mediators, such as histamine, proteolytic enzymes, and chemotactic factors, and the synthesis of newly formed mediators, including prostaglandins and leukotrienes. These mast cell mediators are critical for the development of inflammation and may potentiate the ocular inflammation of EIU by increasing vascular permeability. It is well documented by previous investigators that the inflammatory mediator thromboxane B2 appears in aqueous as early as 1 hr after endotoxin injection. Prostaglandin E2, leukotriene B4, and substance P are significantly elevated between 6 and 72 hr after LPS injection. Then, as a consequence of increased vascular permeability, histologic evidence of inflammation is first noted 3 hr after LPS injection, peaks 24 hr after endotoxin injection, and does not completely resolve until 1 wk later. Furthermore, compound 48/80, a nonimmunogenic mast cell degranulation agent, increases cAMP and protein concentration in the aqueous humor in rabbits, and alters vascular permeability and induces an influx of inflammatory cells in conjunctiva of rats.

During the induction period of EIU in the susceptible Lewis rats, the mean number of mast cell decreased to 74% of normal at 4 hr and to 40% of normal at 8 hr after LPS injection. This decrease in mast cell number is most consistent with mast cell degranulation. There was no significant change in mast cell number during the induction period for EIU in the LBNF1 rats. However, there was a small but significant increase in mast cell number in the BN rats 8 hr after endotoxin injection. One could hypothesize that mast cells migrated into the anterior area during the early induction period for EIU and then were available to degranulate and potentiate ocular inflammation. BN and LBNF1 rats, with low overall mast cell numbers in anterior uvea, are more resistant to EIU. This suggests that mast cell numbers are important to the development of EIU, but are not the sole critical factor involved.

Finally, mast cell degranulation appears to occur in the mid-stage of EIU induction, suggesting that other events may initiate ocular inflammation. A number of potential candidates have been demonstrated by previous investigators. LPS can stimulate cultured ciliary body cells to secrete prostaglandin E2 and express membrane-associated IL-1. LPS also can induce expression of CD11/CD18 (Mac-1), a cell adhesion molecule important for the migration of neutrophils and monocytes to areas of inflammation. Therefore, inflammatory mediators from mast cells may amplify the progressing inflammatory cascade to increase susceptibility to EIU.

Key Words
endotoxin-induced uveitis, inbred rat strains, mast cells.

References