Lipoteichoic Acid as an Inducer of Acute Uveitis in the Rat
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PURPOSE. To examine the capacity of lipoteichoic acid (LTA) to induce intraocular inflammation in the rat.

METHODS. LTAs obtained from Staphylococcus aureus and three different streptococcal species were suspended in saline solution in various concentrations and injected into one footpad of female Lewis rats. The uveitic changes were assessed by conventional clinical and histopathologic procedures, whereas the intensity of inflammation in the anterior chamber (AC) was evaluated by the measurement of protein concentration and cell density in the aqueous humor (AH).

RESULTS. LTA from S. aureus induced a strong intraocular inflammation between 24 and 30 hours after injection. The inflammatory reaction was observed in a dose-dependent manner. At a dose of 15 mg/kg LTA, the protein concentration and cell counts in the AH were 5.6 ± 0.5 mg/ml and 4075 ± 1193 cells/μl, respectively. When LTAs of streptococcal origin were used, cells were undetected in the AH and protein concentration increased only twofold compared with the control group. In pathologic examination, inflammatory cells were found in the AC and posterior chamber only after the injection of S. aureus LTA. In systemic evaluations of the liver, kidney, spleen, heart, lung, gut, brain, joint, and eye performed 6, 24, and 48 hours after the challenge, inflammatory lesions were found only in the eye.

CONCLUSIONS. LTA, especially of S. aureus origin, induces anterior uveitis in the rat. This model may be useful for investigation of Gram-positive bacterial infection and uveitis. (Invest Ophthalmol Vis Sci. 1998;39:1251-1256)

Uveitis in humans remains a field in which pathogenetic mechanisms and uveitogenic antigens are still issues to be addressed. Bacteria are thought to be one of the causes of uveitis; Gram-negative bacteria have been linked to the development of uveitis in inflammatory bowel diseases, ankylosing spondylitis, and Reiter syndrome.1 Gram-positive bacteria also are related to ocular inflammation; streptococcal species have been reported to participate in the pathogenesis of Behcet's disease2 and in uveitis after infection.3 Animal models of uveitis have been developed using various bacterial antigens. Endotoxin-induced uveitis, induced by lipopolysaccharide (LPS) from Gram-negative bacteria, is a widely studied experimental model in animals. Important mechanisms regarding the breakdown of the blood-aqueous barrier and the recruitment of inflammatory cells into the eye have been clarified using this model.1 However, a uveitis model induced by a component specific to Gram-positive bacteria in the rat has not been established.

Lipoteichoic acid (LTA) is a high-molecular-weight amphiphile associated with the cell membrane of Gram-positive bacteria, which consists of a hydrophobic glycolipid moiety and a hydrophilic chain. Many biologic functions of LTA have been described, including activation of complements, the induction of cytokine secretion by macrophages and blood monocytes, and the induction of nitric oxide in macrophages and superoxide radicals in blood monocytes.4 We have developed a novel experimental uveitis model induced by LTA in the rat. This is the first article to show that a component specific to Gram-positive bacteria has the capacity to induce an acute uveitis in the rat.

MATERIALS AND METHODS

Animals

Inbred female Lewis rats, 7 to 9 weeks old and weighing 150 to 200 g, were purchased from Charles River Japan (Atsugi, Kanagawa, Japan). Food and water were provided ad libitum. All animals were treated in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Lipoteichoic Acids

LTAs were obtained as lyophilized powder from Sigma Chemical (St. Louis, MO) with the following specifications: Staphylococcus aureus LTA, lot 75H4048; S. sanguis LTA, lot 72H4049; S. pyogenes LTA, lot 113H4013; and S. faecalis LTA, lot 104H4039. All these reagents were dissolved in sterile saline (0.9%) under aseptic conditions and were used immediately. The amount of LPS in the LTAs was measured using the Limulus test kit (Seikagaku Kogyo, Tokyo, Japan). The assays indicated that the S. aureus LTA contained 207.8 ng LPS/mg LTA; the S. sanguis LTA contained 95.3 ng LPS/mg LTA; the S. pyogenes LTA contained 10.7 ng LPS/mg LTA; and the S. faecalis LTA contained 55.4 ng LPS/mg LTA.

Induction of Uveitis with Lipoteichoic Acid

LTAs were injected into a hind footpad of each animal at a volume of 100 μL. Dose-response studies were performed using increasing concentrations of S. aureus LTA (3.75, 7.5, 15, and 30 mg/kg, in eight eyes). Control animals (eight eyes) received an equivalent volume of saline solution. Aqueous humor (AH) samples were obtained 24 hours after the injection. Time-course experiments were carried out using 15 mg/kg of the same LTA (six eyes in each time period). LTA from streptococcal origin was injected at a concentration of 15...
FIGURE 1. (A) Appearance of rat eye 24 hours after the injection of 15 mg/kg of *Staphylococcus aureus* lipoteichoic acid (LTA). Note the conjunctival injection, iris hyperemia, miosis, and fibrin deposit around the pupil. (B) Fundus appearance of a normal eye. Note the thin diameter of the arteries and veins. (C) Aspect of the fundus 24 hours after the injection of 15 mg/kg of *S. aureus* LTA. Hyperemia of the optic disc and enlargement of the retinal arteries and veins are prominent.

mg/kg, and AH samples were collected after 24 hours (eight eyes each).

**Evaluation of Lipoteichoic Acid–Induced Uveitis**

Intraocular inflammation was evaluated through clinical observation under a microscope (OMS-50; Topcon, Tokyo, Japan) by the determination of total protein concentration and cell density in the AH and by histologic analysis of ocular specimens. Fundus changes were photographically recorded by means of a fundus camera (Genesis; Kowa, Tokyo, Japan). Protein concentration and cell counts in the AH were used as parameters to evaluate inflammatory activity in the dose-response and time-course studies.

**Aqueous Humor Sampling and Protein and Cell Determination in Aqueous Humor Specimens**

The animals received systemic anesthesia by intramuscular injection of 40 mg/kg ketamine hydrochloride (Sankyo, Tokyo, Japan) and 4 mg/kg xylazine (Rompun; Bayer Japan, Tokyo, Japan). Soon after anesthesia was achieved, the AH was obtained by puncturing the anterior chamber using a 25-gauge needle. To avoid clotting, 5% EDTA was added to the AH sample at a 9:1 ratio (final concentration was 0.5% vol/vol). Protein concentration in the AH samples was determined using a protein assay (Bio-Rad Laboratories, Richmond, CA). The determination of cell density was performed with the improved Neubauer hemocytometer under microscope (CH; Olympus, Tokyo, Japan).

**Systemic Evaluation after Lipoteichoic Acid Administration**

Under systemic anesthesia, three rats per time point were killed 6, 24, and 48 hours after an injection of 15 mg/kg *S. aureus* LTA. Immediately, specimens from liver, kidney, spleen, heart, lung, gut, brain (cerebrum, cerebellum, medulla, and pineal gland), joint, and eye were collected and fixed in 4%
paraformaldehyde for 24 hours. All specimens were stained with hematoxylin and eosin and were analyzed in masked fashion by an independent pathologist.

Statistical Analysis
The data are presented as means ± SD. Results were analyzed by means of the Wilcoxon nonparametric rank sum test. The difference between two groups was determined to be significant at \( P < 0.05 \).

RESULTS

Ocular Inflammation Induced by \textit{S. aureus} Lipoteichoic Acid
Clinical signs of uveitis were detected 6 hours after the footpad injection of 15 mg/kg \textit{S. aureus} LTA (three rats) and consisted of miosis and iris hyperemia. Thereafter, inflammatory signs increased, reaching a peak 30 hours after LTA administration. Five days later, only a few scattered cells remained on the lens. The three control animals did not show any inflammatory change in their eyes. Hyperemia of the optic disc and prominent enlargement of arteries and veins were observed in the fundus. The inflammatory changes described earlier were observed in all animals injected with \textit{S. aureus} LTA.

Dose-Dependent Effect of \textit{S. aureus} Lipoteichoic Acid on Intraocular Inflammation
The dose-dependent effect of \textit{S. aureus} LTA on intraocular inflammation is shown in Figure 2. For all tested doses, animals treated with LTA showed significantly higher protein concentration and cell count values than control animals (\( P < 0.0001 \) and \( P < 0.036 \), respectively). Both parameters showed a dose-related increase when 3.75, 7.5, and 15 mg/kg LTA were used. With the 15 mg/kg dose of LTA, the protein concentration and cell counts were 5.6 ± 0.5 mg/ml and 4075 ± 1193 cells/\( \mu l \), respectively. However, after the administration of 30 mg/kg LTA, only the protein concentration increased following the same trend, whereas the cell count remained at that level after the injection of 15 mg/kg LTA.

Time Course of Protein Concentration and Cell Counts in Aqueous Humor after \textit{S. aureus} Lipoteichoic Acid Administration
The time course of protein concentration and cell counts in the AH are illustrated in Figure 3. Six hours after the \textit{S. aureus} LTA injection the protein concentration was already high (\( P < 0.002 \)); afterward it rose progressively and peaked at 30 hours (5.8 ± 1.6 mg/ml; \( P < 0.014 \)). Cells were detected for the first time 12 hours after the LTA administration. Their peak value

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**Figure 2.** Effect of increasing doses of lipoteichoic acid (LTA) on protein concentration and cell counts in the aqueous humor. Samples were obtained 24 hours after \textit{Staphylococcus aureus} LTA injection and are expressed as the mean ± SD of eight samples.
FIGURE 3. Time course of protein concentration and cell counts in the aqueous humor after the administration of *Staphylococcus aureus* lipoteichoic acid. Each point represents the mean value ± SD of six eyes. The animals included for analysis at time 0 were injected with equal volumes of saline.

FIGURE 4. Histologic cross section of an eye 24 hours after treatment with 15 mg/kg of *Staphylococcus aureus* lipoteichoic acid. Inflammatory cells in the stroma and scattered cells in the anterior chamber and vitreous cavity were observed (hematoxylin and eosin stain; original magnification, X50).
also was observed 30 hours after LTA administration (5128 ± 1035 cells/μl). Thereafter both parameters declined, but at 72 hours they still did not reach the base concentration. No cells were detected in control animals.

**Histologic Examination**

Inflammatory changes were observed exclusively in ocular specimens 24 and 48 hours after *S. aureus* LTA administration. Figure 4 shows a histologic section of the anterior segment of an eye obtained 24 hours after LTA administration.

**Aqueous Humor Changes after Treatment with Lipoteichoic Acid from Streptococcal Species**

The ocular inflammation induced by streptococcal LTAs was much weaker than that induced by *S. aureus* LTA. The protein concentrations in the AH obtained from rats treated with streptococcal species *S. sanguis*, *S. pyogenes*, and *S. faecalis* were 0.77 ± 0.26, 0.57 ± 0.46, and 0.51 ± 0.23 mg/ml, respectively. The values were two to three times higher than those found in the control animals (0.25 ± 0.15 mg/ml; P < 0.05). None of the rats showed cells in the AH.

**DISCUSSION**

A new model of uveitis induced by a bacterial antigen, LTA, has been developed in the rat. To rule out contamination by LPS in the antigens used in this study, we checked the presence of LPS in the LTAs by means of the Limulus test. Although the assays showed the presence of endotoxin in LTA, the LPS amount (3.1 μg/kg in an LTA dose of 15 mg/kg) was considerably lower than the LPS dose required to induce uveitis in rats (40 μg/kg). Therefore, we can ascribe the intraocular inflammatory activity observed in our model to LTA or to some active moiety present in LTA. As for the other components of Gram-positive bacteria, peptidoglycan-polysaccharide and its monomer, muramyl dipeptide, were reported to induce intraocular inflammation in the rabbit. Wells et al. reported that peptidoglycan-polysaccharide induced a uveitis and polyarthritis in Lewis rats, but later one of the authors indicated that the inflammation was a side effect of the drug combination used as anesthesia (see Ref. 8). Therefore, no report has appeared on an experimental uveitis in the rat induced by a component of Gram-positive bacteria.

Our results show that many of the characteristics of our model are similar to the endotoxin-induced uveitis model; after a single injection of the antigen, an acute and autolimited breakdown of the blood-ocular barrier and a dilution of the blood vessels develop in the eye. The kinetics of the ocular inflammatory response also share a similar pattern with the endotoxin-induced uveitis model. However, the minimum LTA dose required to produce a supramaximal response is several times higher than the one reported for LPS. Our data show, too, that its uveitogenic power varies according to the source of antigens. LTA obtained from three different streptococcal species raised the protein concentration only modestly and failed to induce a cellular response in the anterior chamber, but the injection of LTA obtained from *S. aureus* resulted in a strong intraocular inflammation. We cannot explain these differences. However, it should be noted that a wide spectrum of biologic effects induced by LTA has been described in the past and that the magnitude and characteristics of some of these biologic activities vary according to the origin of the LTA. Therefore, it is probable that structural differences in the molecular composition of antigens obtained from different bacteria might explain our results.

Although the precise mechanism underlying the development of this uveitis is still unknown, the strong inflammatory response derived from the LTA administration most probably is caused by the release and activity of host-derived mediators, among which cytokines may play an important role. Recently, several publications have shown that LTA induces the production of MIP-1 alpha, interleukin-8, and nitric oxide and the breakdown of the blood-brain barrier. The specific nature of the ocular response is demonstrated by the absence of inflammatory signs in other organs when analyzed by routine histologic techniques. We cannot explain this specificity, which also is observed in the endotoxin-induced uveitis model.

We speculate that LTA causes a selective inflammation in the eye because of the higher sensitivity of the ocular structures to these antigens or the inflammatory mediators that are released systemically. The possible roles of cytokines in LTA-induced uveitis are now under detailed investigation.

Our animal model supports the hypothesis that antigens derived from Gram-positive bacteria should be considered as possible etiopathogenic factors in the development of human uveitis. Gram-positive bacteria are ubiquitous microorganisms, located in the skin and in the respiratory, gastrointestinal, genital, or oral mucosa. They are the cause of acute inflammatory processes, and, in some genetically susceptible hosts, they are the cause of diseases such as acute glomerulonephritis after streptococcal infection, acute rheumatic fever, and uveitis after infection. Further study is needed to elucidate the relationship between LTAs and human uveitis.

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**References**

Matrix Metalloproteinases and Their Inhibitors in Human Vitreous

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PURPOSE. To conduct zymographic analysis to study the matrix metalloproteinases (MMPs) and tissue inhibitor of metalloproteinases (TIMPs) in vitreous samples of patients undergoing pars plana vitrectomy as part of the treatment of vitreoretinal disease.

METHODS. Forty-two vitreous samples were collected at the time of pars plana vitrectomy. Diagnoses included severe (exudative) age-related macular degeneration (AMD) (12), macular hole (10), presumed ocular histoplasmosis syndrome (6), proliferative diabetic retinopathy (PDR) (5), epiretinal membrane (4), vitreomacular traction syndrome (2), macuopneumonia with subretinal hemorrhage (1), central retinal vein occlusion with vitreous hemorrhage (1), and proliferative vitreoretinopathy (1). Gelatin zymography, reverse gelatin-zymography, carboxymethylated transferrin zymography, and sodium dodecyl sulfate-polyacrylamide gel electrophoresis were performed on the liquid vitreous samples to assess for MMP and TIMP activity.

RESULTS. Progelatinase A occurred in all vitreous samples. In addition, a band consistent with TIMP-2 occurred in all samples on reverse zymography. An inhibitor of MMP of a lower molecular weight than TIMP-1 was found in all the samples. A serine proteinase with a broad band around 180 kDa was found in 2 of the 11 AMD vitreous samples. A 75-kDa metalloproteinase was found in several AMD samples, but it was much more abundant in the PDR samples.

CONCLUSIONS. Metalloproteinases and their endogenous inhibitors are present in human vitreous and may be involved in the pathogenesis of PDR and other vitreoretinal diseases. (Invest Ophthalmol Vis Sci. 1998;39:1256-1260)

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