The continuous and quantitative observation of permeability changes of the blood-aqueous barrier in allergic inflammation of the eye

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Permeability changes of the blood-aqueous barrier were studied in the eyes of rabbits subjected to immunologic inflammation. The changes were investigated by slit-lamp microphotometers. The leakage of fluorescein-labeled rabbit serum albumin into the anterior chamber was observed in eyes inflamed by reverse passive Arthus reactions. The permeability of the blood-aqueous barrier changed in biphasic pattern in allergic inflammation; the first phase began 5 min after the antigen challenge and lasted for 1 hr, followed by the late phase at which the dye concentration reached a peak 2 hr after the challenge and then gradually decreased.

Key words: continuous observation, permeability, blood-aqueous barrier, ocular inflammation, allergic inflammation

By a variety of agents, the blood-aqueous barrier is broken down, and serum proteins flow into the aqueous humor through the barrier. The increase of protein concentration in the aqueous humor is associated with the severity of the ocular inflammation.

Anterior chamber taps have been performed for the measurement of protein concentration in the aqueous humor. However, a change of protein concentration cannot continuously be observed. Recently, a method using a slit-lamp microphotometer has been developed for the continuous observation of protein concentration in the anterior chamber. Even by this means, the accurate measurement of protein concentration of the aqueous humor is still very difficult; corneal opacity often interferes.

The present paper offers preliminary data on a new method for the continuous and quantitative measurement of permeability changes of the blood-aqueous barrier by slit-lamp microphotometry, with the use of fluorescein-labeled rabbit serum albumin (FITC-RSA) as an indicator.

Materials and methods

Animals. New Zealand albino rabbits weighing 2.0 to 3.5 kg were employed. Before use, all eyes were examined for the pre-existing diseases.

Antiserum and antigen. Bovine gamma globulin (BGG; Miles Laboratories, Inc., Kankakee, Ill.) was dissolved in phosphate-buffered saline (pH 7.2) at the concentration of 2%. Anti-BGG serum (2.2 mg of antibody N per milliliter) was prepared by repeated immunization of rabbits with BGG in complete Freund’s adjuvant. A normal rabbit serum was used as the control. These sera and the antigen solution were sterilized by passage.
through a 0.22 μm Millipore filter (Millipore Corp., Bedford, Mass.) and stored at −20° C without preservative.

**FITC-RSA.** The rabbit serum albumin fraction (RSA) was obtained by repeated salting out of pooled normal sera in 50% saturated ammonium sulfate. RSA was conjugated with fluorescein isothiocyanate (Sigma Chemical Co., St. Louis, Mo.) according to the method of Riggs et al. and stored at −20° C after sterilization by Millipore filtration.

**Antibody injection.** An intravitreal injection was performed after topical anesthesia with 0.5% proparacaine HCl. The eye was fixed by gently grasping the superior rectus muscle at its insertion with forceps; it was rotated downward. A sharp 27-gauge needle (Termo Co., Ltd., Tokyo, Japan) was then inserted at a slight backward angle 2 mm behind the corneoscleral junction, avoiding the lens. A 0.1 ml amount of anti-BGG rabbit serum was injected into the center of the vitreous. The initial reaction to the trauma of injection had completely subsided within 4 days in all eyes. Just after resolution of the reaction, the challenge was performed by the intravenous injection of 2.5 ml of the BGG solution (50 mg).

**Fluorescence measurement.** The measurement of fluorescein concentration in the anterior chamber of eyes was carried out using a slit-lamp microphotometer. A 0.5 ml amount of FITC-RSA per kilogram of body weight was injected intravenously 30 min before the challenge. The intensity of fluorescence from materials leaking into the anterior chamber was measured by a slit-lamp microphotometer (Hamamatsu I.V. Co., Hamamatsu, Japan), constructed according to the methods of Maurice and Mishima. Our instrument had two microphotometers connected with a recorder to observe both eyes simultaneously and to record data automatically on one chart. The sensitivities of the microphotometer fitted on the instrument were adjusted to the same level with a standard fluorescein solution just before use and checked for their stability just after each experiment.

**Tissue preparation.** Animals were killed by intravenous air injection. The eyes were removed and fixed with 10% formaldehyde. Paraffin sections were cut at 3 μm and stained with hematoxylin and eosin (H&E).

**Results**

The experiments were designed to observe continuously and quantitatively the permeability changes of the blood-aqueous barrier in allergic inflammation of the eye by slit-lamp microphotometry with FITC-RSA as an indicator.

**Concentration of FITC-RSA in the anterior chamber.** Nine animals were employed. Anti-BGG rabbit serum was injected into an eye on one side, and the control eye on the opposite side received the same volume of normal rabbit serum. Just after resolution of the initial reaction to the trauma of intraocular injection, a challenge was performed by the intravenous injection of antigen. FITC-RSA was also injected intravenously 30 min before the challenge.
Fluorescence was not demonstrable in the anterior chamber of either eye until 5 min after the antigen stimuli. In the anti-BGG-injected eye, the dye appeared in the anterior chamber through the pupil about 5 min after the challenge. The fluorescein intensity increased over the next 15 min but soon reached a plateau. The dye concentration was maintained for about 50 min, after which it again began to increase remarkably and then gradually reached a peak 2 hr after the challenge (Fig. 1). After the passage of the peak, the concentration gradually decreased, and the dye had completely disappeared from the anterior chamber by the next day. Meanwhile, FITC-RSA was undetectable in the anterior chamber of control eyes throughout the experiment.

**Concentration of FITC-RSA in circulating blood.** Two animals were bled from an auricular vein at 30 min intervals after intravenous injection of FITC-RSA. The sera were then separated by centrifugation at 2500 × g for 10 min. The fluorescein intensity of these sera was measured by a slit-lamp microphotometer. Ratios between dye concentrations at 30 min and those observed at other times were calculated. Fig. 2 shows changes of the ratios in two animals. The dye concentration of blood in peripheral vein decreased steeply during the first hour, moderately during the next hour, and then gradually thereafter.

**Ratio of FITC-RSA concentration in the anterior chamber and to that in circulating blood.** The amount of FITC-RSA that leaks into the aqueous humor depends on the concentration of the dye in the circulating blood, which varies as shown in Fig. 2. Therefore the ratio of dye concentration found in the anterior chamber to that found in the circulating blood was considered to be a more appropriate indicator for permeability changes of blood-aqueous barrier than the concentration of the dye in the anterior chamber itself.

In nine animals, the dye concentration in the circulating blood was measured 90 min after the FITC-RSA injection, and concentrations for other intervals were calculated with the above values and the standard curve (Fig. 2). The time course of the ratio of dye concentration in the anterior chamber to that observed in the circulating blood is shown in Fig. 3.

**Pathological observation.** Histopathological examination was performed on intraocular tissues 30 min after the antigen challenge.

In the antibody-injected eyes, marked edema was observed in the interstitial tissues.
Fig. 3. Relative concentrations of FITC-RSA in the anterior chamber of eyes during allergic inflammation in rabbits. The zero time indicates the antigenic challenge. The points plotted represent mean values of nine rabbits.

Discussion

The purpose of our experiments was to develop a method which would allow us to observe continuously and quantitatively the fluorescein intensity in the anterior chamber by a slit-lamp microphotometer, placing emphasis on the following three points: (1) simultaneous observation of both eyes of a rabbit, (2) induction of standardized inflammation in one eye, and (3) use of a stable indicator which enters the anterior chamber of normal rabbit eyes only in trace quantities and is not irritating.

In order to meet the first requirement, two microphotometers were used, connected so as to record data for both eyes on a chart. With this instrument, both eyes can be observed simultaneously. Ocular inflammation was induced by an immune reaction: the so-called reverse passive Arthus reaction. Eyes were sensitized by an intravitreal injection of anti-BGG rabbit serum. After resolution of the inflammation that resulted from the trauma of injection, an intravenous challenge of antigen caused a moderate and easily standardized inflammation in eyes which had received antibody at an earlier time. As an indicator, we used FITC-RSA. Although fluorescein sodium itself passes through the blood-aqueous barrier of a normal rabbit, FITC-RSA does not. It was not detected in the anterior chamber of eyes injected with rabbit serum.

In all, permeability changes of the blood-aqueous barrier were observed in nine rabbits. The fluorescence intensity in the anterior chamber of inflamed eyes appears to change in two steps as shown in Fig. 3. Five minutes after the intravenous challenge of antigen, the dye appeared in the anterior chamber through the pupil. The increase of dye concentration was steep during the first 30 min and gradual during the following 30 min, after which the fluorescence was again increased markedly in the anterior chamber. The concentration reached a peak 2 to 3 hr after the stimuli and then gradually decreased. The results suggest that permeability changes of the blood-aqueous barrier of the rabbit may be biphasic in the case of...
inflammation produced by reverse passive Arthus reactions.

Di Rosa and colleagues\(^9\) have shown that the vascular permeability in rat feet by injection of carrageenin also changes in a biphasic pattern. The immediate phase of the increased permeability is observed during the first hour, and is considered to be mediated by histamine and serotonin. Therefore we examined effects of antihistamines. Promethazine (8.5 mg/kg), an H-1 receptor blocker, was administered intravenously in eight rabbits 5 min before and 60 min after the antigen challenge. As shown in Fig. 5, the drug suppressed dye leakage into the anterior chamber throughout the period of experiments. After 60 min, however, dye permeated the blood-aqueous barrier. As shown in Table I, there was no significant difference of the increase of dye concentration in the anterior chamber after 90 min between the two groups of animals. The drug markedly suppressed vascular responses during the first hour, but the later responses were not
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Fig. 5. Effects of promethazine on permeability changes of the blood-aqueous barrier in allergic inflammation of eyes. Promethazine was administered as described in the text. The antigen was injected intravenously at zero time. All values are the mean ± S.E. The p values were calculated by Welch’s t test.

Table 1. Effect of promethazine on the permeability change during the late phase of ocular inflammation of our model

<table>
<thead>
<tr>
<th></th>
<th>90 min</th>
<th>120 min</th>
<th>180 min</th>
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<tbody>
<tr>
<td>Untreated (n=8)</td>
<td>17.9 ± 3.9</td>
<td>23.0 ± 5.0</td>
<td>27.3 ± 4.6</td>
</tr>
<tr>
<td>Promethazine-treated (n=7)</td>
<td>9.2 ± 2.1</td>
<td>13.5 ± 3.7</td>
<td>22.9 ± 5.3</td>
</tr>
<tr>
<td>p value*</td>
<td>&lt;0.05</td>
<td>n.s.</td>
<td>n.s.</td>
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ΔF = difference of relative dye concentration between indicated times and 60 min after the antigen challenge; n.s. = not significant; n = number of animals.

*p values were calculated by Student’s t test.

affected at all. This result indicates that permeability changes of the blood-aqueous barrier are biphasic and that the first phase of the inflammation may be partly mediated by histamine.

Wilhelm and Manson10 also reported biphasic changes of vascular permeability after thermal inflammation of rabbit skin. In their experiments, the first phase began a few minutes after the stimulus and lasted for 30 min. The time course of vascular leakage is the same as in the two experiments, but there is a lag in the first response in ours. The lag is due to the delay in observing the dye concentration at the center of the anterior chamber. Since it takes several minutes for dye to appear in the anterior chamber after the leakage through small blood vessels in ciliary and iridic processes, the increased vascular permeability may well have occurred a few minutes after the intravenous challenge. Finally, after ultraviolet light injury, vascular permeability also changes biphasically.11 However, the late phase slowly develops, reaching a peak in 23 to 26 hr. On the contrary, the peak of the last phase in our experiments occurred in 2 to 3 hr. This suggests that the change in vascular permeability during the late phase of ultraviolet light injury differs from that in allergic and thermal inflammation.

By the method described above, continuous and quantitative observation of permeability change in the blood-aqueous barrier is possible. As a result, effects of pharmacological agents on ocular inflammation are being examined in detail by this technique.

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