A Hapten Model of Topically-Induced Ocular Anaphylaxis in the Rat

A hapten (DNP) model of topically induced ocular anaphylaxis has been developed. Rats immunized with DNP-Ascaris were skin-tested with DNP-bovine serum albumin (DNP-BSA) and Evans blue and challenged topically with varying amounts of di-DNP-lysine. The degree of clinical conjunctival edema was assessed, and eye tissues were evaluated histologically. Clinical conjunctival edema and histologic mast cell degranulation increased with higher concentration of di-DNP-lysine. In general, rats with positive skin tests showed more clinical conjunctival edema and more mast cell degranulation than those with negative skin tests. Three other groups of rats with positive skin tests to the DNP-BSA were injected intravenously with 125I-BSA and challenged topically with di-DNP-lysine. Retention of 125I-BSA in ocular adnexa and in globes was higher in di-DNP-lysine- than in PBS-challenged eyes. The hapten model simulates the ocular component of human hay fever in that ocular anaphylaxis is induced in immunized rats by topical challenge with antigen alone. Invest Ophthalmol Vis Sci 28:264-269, 1987

We demonstrated previously that ocular tissues participate in systemic anaphylaxis elicited by intravenous challenge with antigen in immunized rats.1 Ocular anaphylaxis has also been elicited by local injection of antigen into eye tissues of immunized rats2 and by local injection of anti-IgE in unimmunized rats.3 Since allergic reactions in the human conjunctiva are presumably induced by topically acquired antigen, the ocular component of human hay fever was more closely simulated by models in which rats were topically challenged with antigen after immunization with egg albumin or infection with Nippostrongylus brasiliensis.5 However, the rat conjunctiva appears to be relatively impermeable to topically administered antigens of even moderate molecular weight, such as egg albumin and worm extract. In the above-mentioned topical models,4,5 ocular anaphylaxis was elicited only by application of antigen to eyes topically pretreated with dithiothreitol (DTT), a mucolytic agent. Iso et al6 encountered similar problems in inducing ocular anaphylaxis by topical application of egg albumin to passively immunized rat eyes, but succeeded in inducing ocular anaphylaxis by intravenous administration of egg albumin. Various aspects of ocular anaphylaxis have also been studied in guinea pig models by Dwyer et al,7 Kathami et al,8,9 and Woodward et al.10 In the current study, ocular anaphylaxis was induced in rats without DTT, by topical application of di-DNP-lysine, a compound of relatively low molecular weight, to eyes of rats immunized with DNP-Ascaris. Previous studies have demonstrated that crude Ascaris suum extract is a potent immunogen11,12 and that the dinitrophenylated derivative of the extract (DNP-Ascaris) evokes production of DNP-specific IgE antibodies in the rat.13 Ocular anaphylaxis was assessed by clinical observation, evaluation for histologic mast cell degranulation, and determination of 125I-BSA retention in ocular tissues.

Materials and Methods

Antigens

Ascaris suum extracts were prepared according to Strejan and Campbell's method.12 Dinitrophenyl (DNP) groups were added to Ascaris extracts by the method of Eisen et al.14 Dinitrophenylated bovine serum albumin (BSA) was prepared by a similar procedure. Crystalline N,N'-di-2,4-DNP-L-lysine (Sigma Chemical; St. Louis, MO), MW = 478.4, was dissolved in phosphate-buffered saline (PBS), pH 7.6, immediately before use. Because of poor solubility, concentrations greater than 1.00 mg/ml were not tested.

Immunization

Male Sprague-Dawley rats weighing approximately 250 g were injected intraperitoneally with 1 ml of a...
Table 1. Characteristics of rat groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of rats</th>
<th>Immunized</th>
<th>Challenge level of di-DNP-lysine (mg/ml)</th>
<th>Skin-test range</th>
<th>Interval between antigen challenge and death (min)</th>
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</thead>
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<tr>
<td>1</td>
<td>9</td>
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<td>0.01</td>
<td>0-3+</td>
<td>60</td>
</tr>
<tr>
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<td>0.10</td>
<td>0-3+</td>
<td>60</td>
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<tr>
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<td>1.00</td>
<td>3+</td>
<td>15</td>
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<tr>
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<td>1.00</td>
<td>3+</td>
<td>60</td>
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<tr>
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<td>3+</td>
<td>60</td>
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<tr>
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<tr>
<td>7</td>
<td>3</td>
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<td>1.00</td>
<td>no test</td>
<td>60</td>
</tr>
</tbody>
</table>

suspension containing DNP-Ascaris (200 μg) and alum (20 mg).

All procedures using rats conformed to the ARVO Resolution on the Use of Animals in Research.

Active Cutaneous Anaphylaxis (Skin Test)

On day 13, immunized rats were tested with intradermal injection (100 μl) of DNP-BSA solution (100, 10, 1 μg/ml, diluted in PBS), followed immediately by intravenous injection of 1 ml 1% Evans blue dye solution. Reactions were scored 30 min later by measuring the diameter of the blue skin reactions; lesions with a diameter greater than 4 mm were considered positive. The reactions were scored on a scale of 0 (no injection site positive) to 3+ (all DNP-BSA injection sites positive). Di-DNP-lysine did not elicit positive skin tests in immunized rats at the concentrations used for conjunctival challenge (1.00, 0.10, and 0.01 mg/ml).

Histologic Evaluation of Eye Tissues

On day 14, 34 immunized rats were assigned to one of three groups (1, 2, and 3) for topical conjunctival challenge with different concentrations (1.00, 0.10, and 0.01 mg/ml) of di-DNP-lysine (Table 1). All groups included both skin-test negative (0) and positive (1+-3+) rats. Skin-test results were evenly distributed in the three groups. Prior to challenge, all rats were lightly anesthetized with ether and given 35 mg/kg pentobarbital intraperitoneally. Rats were challenged by topical application of 10 μl di-DNP-lysine to the experimental eye. One hour after challenge, the rats were assessed clinically and killed by exsanguination from the neck arteries. Orbits were exenterated and tissues processed as previously described,1 one micrometer of Epon-embedded sections were stained with alkaline Giemsa.1 Mast cells were counted in 30 subepithelial fields, 10 fields in each of three tissue sections, obtained at least 15 μm apart. Mast cells, identified as previously described,1 were classified as granulated, containing all purple granules or fewer than four pink granules, or degranulated, containing four or more pink granules.

Retention of Radioiodinated Bovine Serum Albumin (125I-BSA) in Eye Tissues

Retention of 125I-BSA in ocular tissues was assessed using a modified Byars and Ferraresi method.15 BSA solutions were labeled with 125I according to the method of Fraker and Speek.16

On day 14, 18 immunized rats scored as 3+ on the skin test were subdivided into three groups (4, 5, and 6) (Table 1) and injected intravenously with 35 mg/kg pentobarbital and 3 X 106 CPM 125I-BSA in 1 ml saline. All animals were subsequently challenged with 10 μl di-DNP-lysine (1.00 mg/ml) applied to one eye and 10 μl PBS to the fellow eye. Rats were assessed clinically and killed 15 min (group 4), 30 min (group 5), and 60 min (group 6) after challenge. Blood samples were collected by cardiac puncture, and the animals were then perfused with 60 ml PBS injected into the left ventricle. The inferior vena cava was divided below the diaphragm to allow intravascular fluid to escape. After perfusion, orbits were exenterated, and globes (including bulbar conjunctivas) were separated from the adnexa. All tissues were weighed on a Mettler H 20 balance (Mettler, Highstown, NJ). The amount of radioactivity present in tissues and serum was determined with a gamma counter. Tissue counts in each rat were

Fig. 1. Rat undergoing ocular anaphylaxis at 1 hr (clinical reaction of 3+) in the right eye. The left eye received PBS for control.
Fig. 2. Di-DNP-lysine dose response in rats with positive and negative skin tests. Unshaded, PBS-treated eyes; shaded, di-DNP-lysine-treated (antigen-challenged) eyes. Each point represents a value obtained from an individual rat; the height of the bar corresponds to the median value. Clinical edema in skin-test positive rats (A) and skin-test negative rats (B); mast cell degranulation in skin-test positive rats (C) and skin-test negative rats (D).

Clinical Evaluation

Conjunctival edema of the lower eyelid was assessed under a dissecting microscope (magnification × 3) at different time points after challenge and rated on a scale of 0 (normal conjunctiva) to 3 (maximal observed edema).

Toxicity Test

Two groups of unimmunized rats (groups 7 and 8) served as a control for toxicity. Rats in group 7 were challenged topically with 10 μl di-DNP-lysine (1.00 mg/
ml) and killed at 60 min. Eye tissues were processed to assess histologic mast cell degranulation. Rats in group 8 were challenged topically with 10 μl di-DNP-lysine (1.00 mg/ml) immediately after intravenous injection of 125I-BSA. The rats were assessed clinically and killed 60 min after challenge. Eye tissues were evaluated for 125I-BSA retention.

**Statistical Analysis**

The amounts of radioactivity in eye tissues and the cell counts were compared by a one-tailed Mann-Whitney U test. Rank correlations (Spearman’s rho) were calculated using the RS/1 software (BBN Research Systems, Cambridge, MA). A probability of 0.05 or less was considered significant.

**Results**

**Histologic and Clinical Evaluation**

All rats in groups 1, 2, and 3 were assessed 60 min after challenge; clinical and histological findings were compared.

Skin-test positive rats had significant clinical edema (Fig. 1) following challenge with di-DNP-lysine at 1.00 mg/ml (group 3; P < 0.01) and 0.10 mg/ml (group 2; P < 0.01) levels, but not at the 0.01 mg/ml level (Fig. 2A). Skin-
In skin-test positive animals a significant increase in mast cell degranulation was observed in eyes challenged with di-DNP-lysine at 1.00 mg/ml (group 3; $P < 0.05$) but not at 0.10 (group 2) or 0.01 mg/ml (group 1) levels (Fig. 2D).

Skin-test rating was significantly correlated with clinical assessment ($rho = 0.74; P < 0.01$) (Fig. 3A) and with mast cell degranulation ($rho = 0.90; P < 0.01$) (Fig. 3B). Mast cell degranulation was also significantly correlated with clinical assessment ($rho = 0.65; P < 0.05$) (Fig. 3C).

Retention of $^{125}$I-BSA in Eye Tissues

Skin-test positive rats (all 3+) had significantly increased $^{125}$I-BSA accumulation in the adnexa of eyes topically challenged with DNP-bis-lysine at 15 min (group 4; $P < 0.05$), 30 min (group 5; $P < 0.01$), and 60 min (group 6; $P < 0.01$) after challenge (Fig. 4A). The $^{125}$I-BSA retention in adnexa did not differ significantly among the 15, 30, and 60 min time points. Significant retention of $^{125}$I-BSA was also found in globe specimens (globe and bulbar conjunctiva) obtained from antigen-challenged eyes at 30 ($P < 0.01$) and 60 min ($P < 0.01$), but not at 15 min (Fig. 4B). Furthermore, conjunctival edema was significantly increased at 30 ($P < 0.01$) and 60 min ($P < 0.01$) (Fig. 5). Clinical edema and $^{125}$I-BSA retention were significantly cor-

**Fig. 5.** Clinical assessment at different time points after topical challenge with di-DNP-lysine.

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test negative animals in these groups did not have statistically significant clinical edema (Fig. 2B).

Eye tissues in skin-test positive rats showed a significant increase in mast cell degranulation in eyes challenged with the 0.01 (group 3; $P < 0.01$), 0.10 (group 2; $P < 0.01$), and 1.00 mg/ml (group 1; $P < 0.01$) concentrations of di-DNP-lysine (Fig. 2C). In
related (rho = 0.68; P < 0.05) in groups 4, 5, and 6 combined (Fig. 3D).

Toxicity Test

Challenged eyes in group 7 had no clinical or histologic signs of anaphylaxis. Rats in group 8 showed no evidence of clinical edema or increased 125I-BSA uptake in di-DNP-lysine challenged eyes.

Discussion

Contrary to previous topical models,4,5 which required treatment with DTT prior to topical challenge, topically applied antigen (di-DNP-lysine), in the present hapten model, appears to penetrate the conjunctival epithelium without DTT pretreatment. The ability to elicit ocular anaphylaxis by application of a substance that does not require pretreatment with DTT makes possible pharmacologic and immunologic modulation studies on ocular anaphylaxis without the need to control for possible effects of DTT on topically applied drugs or on tear immunoglobulins. By skin-testing the rats prior to ocular challenge, it was possible to assess each rat's state of immunity to hapten. A positive skin test (1+–3+) provided a fairly good indication that the eye would undergo some degree of anaphylaxis after topical di-DNP-lysine challenge. Conversely, a negative skin test (0) predicted a low grade or no anaphylactic reaction after challenge.

In addition to clinical and histologic evaluations, ocular anaphylaxis in the hapten model was assessed by measuring retention of 125I-BSA in ocular tissues. Retention of 125I-BSA in ocular adnexa is positively correlated with clinical edema rating and appears to offer a more precise and objective means of assessing conjunctival edema than does clinical observation. The clinical observation that antigen-challenged eyes showed some swelling of the bulbar conjunctiva was confirmed by a significant increase in 125I-BSA retention at 15 min, the retention in globe specimens (globe and bulbar conjunctiva) obtained from antigen-challenged eyes at 30 and 60 min. However, although the adnexa had a significantly increased 125I-BSA retention at 15 min, the retention in globe specimens at that time point was not significantly elevated. This lack of significance may be explained by the small number of rats in the 15-min group and the lower level of 125I-BSA accumulation per mg tissue in globe specimens than in adnexa.

Like our previous topical rat models,4,5 this hapten model demonstrated significant levels of mast cell degranulation in the absence of clinical edema in some rats. This information may have clinical implications since it suggests that ocular allergy caused by conjunctival mast cell degranulation may be present in patients with itchy eyes that appear normal upon examination.

Key words: DNP, hapten model, ocular anaphylaxis, skin test

References