

Experimental allergic conjunctivitis in inbred guinea pig strains with high respectively low bronchial allergic reactivity

L. LUNDBERG,¹ C. BERTELSEN,¹ A. ZAVARO,^{2,4} Z. SAMRA³ and D. SOMPOLINSKY^{3,4,5}

¹Institute of Medical Microbiology, University of Copenhagen, Denmark; Departments of ²Ophthalmology and

³Microbiology, Assaf Harofeh Medical Center, Zerifin; ⁴The Sackler Medical School, Tel Aviv University, Ramat Aviv; and

⁵Rapoport Laboratories for Microbiology, Bar Ilan University, Ramat Gan, Israel

Inbred guinea pigs selected for high (IMM/S) respectively low (IMM/R) responsiveness to ovalbumin (OA) as measured by induced respiratory anaphylaxis, were investigated for atopic immune responses of their conjunctival mucosa. IMM/S animals sensitized either by inhalation of OA, or by instillation of antigen into the conjunctival sac, developed regularly an acute ocular inflammatory response to topical (conjunctival) challenge with the allergen. A minimum of 1 µg OA dropped repeatedly into the conjunctival sac was enough for both ocular and systemic sensitization of the animals, but the minimal dose of effective challenge was considerably higher. In IMM/R animals, ocular hypersensitivity was not achieved by inhalation of OA, but after topical administration of the antigen some of the IMM/R strains could be challenged to ocular anaphylactic responses of the same intensity as observed in IMM/S animals. The conjunctivae of both IMM/S and IMM/R animals could be sensitized passively by intraperitoneal injection of guinea pig sera containing homocytotropic antibodies to OA, but topical administration of such sera had no effect.

Key words: guinea pigs, inbred strains; hypersensitivity, immediate; immunization; ovalbumin; passive transfer of immunity.

Accepted for publication 19 September 1986

CLINICAL ASPECTS

Inbred guinea pig strains could be divided into three groups according to their responsiveness to bronchial and ocular administration of allergen (ovalbumin). One group developed high bronchial and conjunctival allergic reactivity; another group had low bronchial and low conjunctival reactivity; and a third group had low bronchial reactivity although the conjunctival anaphylactic reactions were of the same degree as in the first group. The guinea pigs refractory to inhalations but sensitive to conjunctival administration of ovalbumin may prove to be of value as animal models of vernal conjunctivitis, a disorder usually considered to be a type-I hypersensitivity condition. In subtropic areas where this disorder is endemic it is not associated with systemic atopy.

Chronic allergic disorders of the human eye (e.g., vernal keratoconjunctivitis) are generally assumed to be type-I hypersensitivity conditions (2, 26-28). Vernal keratoconjunctivitis has a worldwide distribution but is particularly widespread in the Middle East (23). Neither familiar clustering nor association with other allergies has been observed in patients from the Middle East (23, 26,

28). Whether sensitization of the ocular tissue in vernal conjunctivitis is due to topical contact with the allergen or to its introduction by other routes has not been established, but in contact lens papillary conjunctivitis, a disorder with similar clinical and immunological findings, the conjunctivae are obviously immunized by direct contact with the presumed antigen (4, 10).

Several investigators have used guinea pigs as experimental models for ocular allergy. In most studies, sensitization was performed by intradermal injection of the antigen (3, 11, 13), but Khatami et al. (18, 19) have recently demonstrated that ocular hypersensitivity can as well and as effectively be obtained by repeated instillations to the conjunctival sac. In all studies reported, sensitization was achieved with antigen doses probably unphysiologically high for the human situation (0.6–6.0 mg protein per application). Inconsistent results have been reported (13), possibly due to genetic heterogeneity of the animal stocks.

In the present study, conjunctival hypersensitivity to ovalbumin (OA) was induced in guinea pigs either by inhalation or by topical application to the conjunctival sac. The minimal doses for hypersensitization and for effective provocation of an inflammatory process were determined. The animals were inbred and selected for high (IMM/S) or low (IMM/R) responsiveness to OA-induced respiratory anaphylaxis (20). The genetic homogeneity of the animal strains has previously been demonstrated by skin grafting and biochemical studies (29). We attempted now to clarify whether or not atopy of the respiratory tract and the outer eye is linked.

MATERIAL AND METHODS

Animals. The guinea pigs used in this study were selectively bred for either high (IMM/S strains 740 and 209) or low (IMM/R strains 201-4b, 201-16 and 203 and line 201-03 (F = 17)*) responsiveness to OA as expressed by the respiratory reaction to repeated inhalation with aerosolized OA in saline.

Antigens. OA, grade 5 (cat. No. A5503) was purchased from the Sigma Company, St. Louis, Mo.

Methods of immunization. a) By inhalation: once

a week for 6 weeks, the animals were exposed to an aerosolized solution of 0.5% OA in saline (20). b) By topical instillation: antigen solution was dropped untraumatically into the conjunctival sac on 3 or 4 consecutive days each week during 2-week periods, with an interval of 1 week between the periods. Doses of 5 mg were given in 25 μ l and smaller doses in 10 μ l. The collateral eye was similarly treated with saline. c) Systemic passive immunization: guinea pig serum (3 ml) containing homocytotropic antibodies to OA was injected intraperitoneally. Challenge as indicated under Results. d) Topical passive immunization: as indicated under Results.

Titration of homocytotropic antibodies. Levels of homocytotropic IgG₁ (heat-stable) and IgE (heat-labile) antibodies were determined as described (24). The PCA titer was defined as the reciprocal value of the highest serum dilution producing a skin reaction of ≥ 5 mm diam.

Challenge for ocular anaphylaxis. OA (5 mg in 25 μ l or less in 10 μ l) was untraumatically instilled into the conjunctival sac. In some studies, the challenge was given repeatedly, as indicated (Fig. 2). Grading was performed after 15–25 min but the animals were observed for 1–2 h.

Anaphylactic reactions

a) Bronchial provocation test. The inhalation was performed and graded in a 0–5 scale (G) as previously detailed (20). The time in minutes (T) for the first clear signs of respiratory distress was also noted.

From T and G an anaphylactic score was defined arbitrarily as $S = \frac{4 \cdot G}{T}$. According to this formula, immunized IMM/S animals consistently obtained a score of > 1 and IMM/R animals a score of < 0.5 .

b) Ocular provocation test. The symptoms were graded as follows: 0: no reaction; 1: slight conjunctival hyperemia; 2: marked conjunctival hyperemia and vasodilation; 3: as 2 but in addition chemosis particularly of the canthal and upper fornical conjunctiva; 4: strongly

* Animal families are designated strains or lines if they are obtained by brother $\times$ sister matings in more than or less than 20 generations (F), respectively.

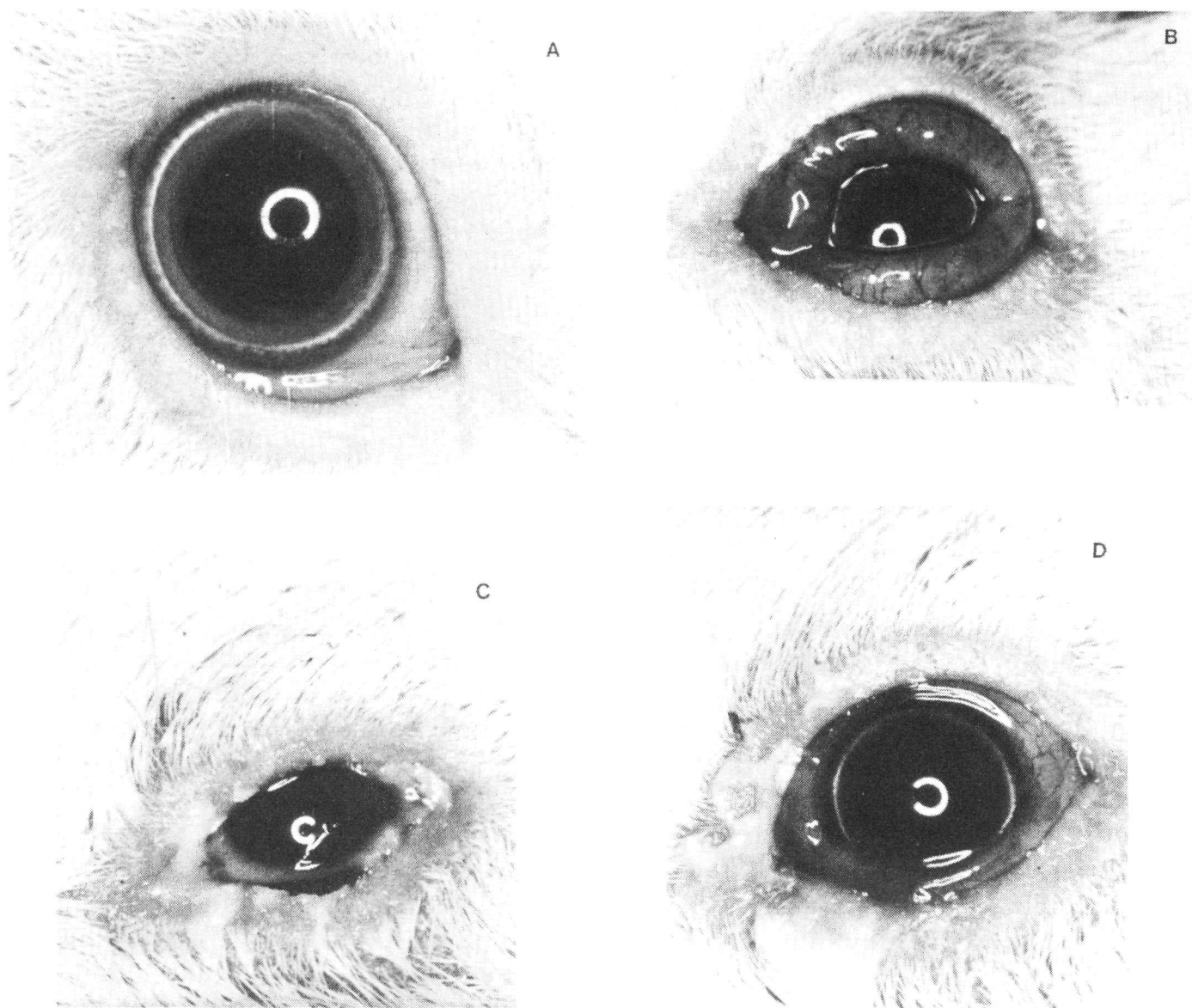


Fig. 1. Ocular anaphylaxis in sensitized guinea pigs challenged with 5 mg ovalbumin dropped into the conjunctival sac.

A. Control eye; B. Eye with a grade-3 response; C, D. Sticky mucous discharge.

expressed conjunctival hyperemia and severe edema of the bulbar conjunctiva, with excess tearing and a sticky, milky discharge (Fig. 1). The bulbar conjunctival edema was in many cases so pronounced that the cornea was partly covered; 5: as 4 but in addition conjunctival diapedesis bleeding. Reactions of grade 4 or 5 were associated with violent attempts to rub the eye, excess urination and defecation, and in some cases forced respiration and even an occasional cough.

c) Skin test. Evaluations 20 min and 24 h after intradermal injections of 1 μ g and 10 μ g OA in 0.1 ml saline. Local infiltration with an

average diameter of ≥ 5 mm was recorded as positive.

In vitro demonstration of antibodies to ovalbumin

Serum levels of anti-OA IgG were monitored by ELISA technique. The wells of microtiter plates were coated with 10 ng/100 μ l OA. After postcoating (BSA), the samples were applied. The detecting system was rabbit anti-guinea pig IgG (γ -chain) and phosphatase-conjugated goat anti-rabbit IgG. A pool of sera from naive guinea pigs was monitored as a negative control. The highest dilution of the

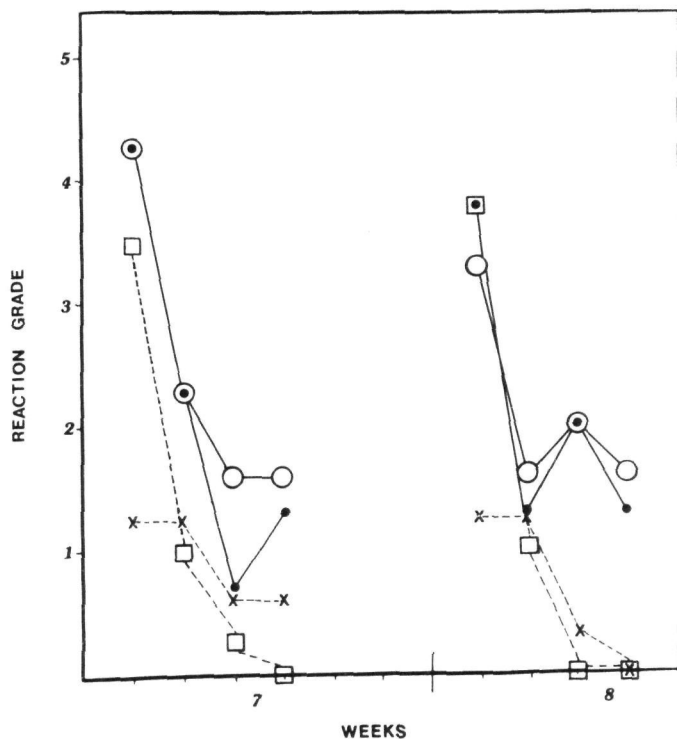


Fig. 2. Relative ocular responses to repeated topical instillations of 1 mg ovalbumin. No response during the first 6 weeks.

- 3 IMM/S animals (strain 740).
- 3 IMM/R animals (strain 203).
- 4 IMM/R animals (line 201-03).
- x 3 IMM/R animals (strain 201-4b).

The figure indicates the mean reaction of each group on different days. On the first day with positive reactions the variability was as follows: all 740 and 203 animals with grade 4-5 reactions; 201-03 animals with grade 3-4 reactions; and 201-4b animals with grade 0-2 reactions. Four 209 animals (IMM/S) reacted as the 740 strain and four 201-16 animals (IMM/R) as the 201-4b strain. For technical reasons these results are not included in the figure.

samples giving a reading at 405 nm of > 0.1 above the corresponding dilution of the negative control was the anti-OA titer.

Statistical methods

The reaction grades of different animal groups were compared by Fisher's exact test.

RESULTS

Sensitization by inhalation of OA

Seven IMM/S animals (strain 209) were sensitized to OA by inhalations (see Material and Methods). They were challenged with 5 mg OA in saline dropped into the right conjunctival sac 7 days after

the last inhalation. All the animals showed a marked ocular anaphylactic response to OA, with chemosis which covered the cornea almost entirely, a mucous sticky discharge, and sporadic diapedesis bleeding (reactions of grades 4 and 5). After 30-45 min the inflammation faded and disappeared after 1-2 h. No macroscopic late reactions were seen. In a similar experiment with 10 IMM/S animals of strain 740 which were challenged topically 6 weeks after the last inhalation of OA, quite similar results were obtained although the reaction grade varied between 2 and 4. However, none of four IMM/R animals showed any reactions to topical challenge with OA, proving that they had not been sensitized by inhalation of OA. Three of these animals (strain 201-4b) remained refractory even when the topical challenge with OA was repeated during several 2-week periods, but one of them (strain 203) showed, starting from the second challenge period, strong reactions (grades 3-5) on the first challenge day of each week, with much weaker reactions the following days. These results indicated that only in the IMM/S-animals were the outer eyes sensitized by inhalation of OA, and that the IMM/R animals were heterogenic in their reaction to repeated topical administration of OA.

Topical immunization with OA

Next, topical immunization with OA was attempted in naive IMM/S (strains 209 and 740) and IMM/R (strains 203, 201-4b, 201-16 and line 201-03) animals. These animals differ with respect to their immune response to synthetic polypeptides (21) and their class-II MHC-tissue types (9). In this experiment, 1 mg OA in saline was dropped into the conjunctival sacs of the right eyes during several 2-week periods. Ocular reactions occurred for the first time during the third period and were rather intensive and associated with general malaise in almost all the 209, 740, 203 and 201-03 animals, but the 201-4b and 201-16 animals remained refractory throughout (Fig. 2). During the days after a strong ocular response, the reactivity was strikingly reduced (Fig. 2). The ELISA anti-OA titers of sera from these animals are indicated in Table 1. The IMM/S animals were the best and the 201-4b animals

the weakest antibody producers. By PCA individual titers of 100–500 for IgG₁ antibodies were obtained in sera from 740 and 203 animals. IgE antibodies were not demonstrated, and 201–4b sera were not tested by PCA.

Minimal dose of antigen for sensitization

Two groups of IMM/S animals were immunized with either 10 µg (7 animals) or 1 µg (4 animals) of OA administered topically to their

right conjunctival sacs. This treatment was repeated during seven 2-week periods of eight immunizations each, but no ocular response was observed. The eyes were then challenged with 5 mg OA in saline, and all the animals developed grade-4 reactions. The next day, the left eyes were challenged in the same way, and they also developed grade-4 reactions. The same day, five of the animals were challenged by inhalation of 0.5% OA, and all responded (anaphylactic scores of 1–2.5). Four other animals were tested intradermally with OA, and all showed immediate but no late reactions. The ELISA anti-OA titers for pools of serum obtained before challenge were 1280 for the group immunized with 10 µg and 80 for the group immunized with 1 µg OA.

The minimal dose for effective sensitization was determined on three groups of 5–7 IMM/S animals (Table 2). Eight topical instillations of 1 µg OA were not, but 16 doses were effective in sensitizing the animals so that local inflammation and general malaise were elicited by challenge with 5 mg OA. Lower doses (0.01

Table 1
Antibodies to ovalbumin in response to repeated instillations of 1 mg ovalbumin to the conjunctival sac

Animal stock	Antibodies, titer ^a	
	Time of immunization	
	1 month	4 months
740 (IMM/S) ^b	160	5120
203 (IMM/R) ^b	40	1280
201-03 (IMM/R) ^b	40	1280
201-4b (IMM/R) ^c	10	320

^a The reciprocal of the highest serum dilution with a positive ELISA reaction. ^b Serum pool from three guinea pigs. ^c Serum pool from four guinea pigs.

Table 2
Minimal effective dose for topical immunization of IMM/S guinea pig eyes with ovalbumin

Guinea pig No.	Immunization dose (μg)	Immunization periods ^a before challenge					Respiratory responsiveness ^c
		Ocular responsiveness ^b					
		1	2	3	4	6	
382	1.0	1				3	1.6
409	1.0	0				4	2.7
398	1.0		5			4	5.3
420	1.0		5			5	3.2
336	1.0			2		4	1.1
422	1.0			5		4	10.0
348	1.0					4	0.9
383	0.1		0			0	0
421	0.1		0			0	0
399	0.1			0		3	4.0
407	0.1			0		0	0
369	0.1					0	0
408	0.01				0	0	0
414	0.01				0	0	0
332	0.01					0	0
370	0.01					0	0
423	0.01					0	NT

^a Two-week periods of eight immunizations with a pause of 1 week between the periods. ^b Ocular anaphylactic responsiveness in a scale of 0–5 grades. ^c Respiratory anaphylactic responsiveness in scores (see Material and Methods). NT = not tested.

and 0.1 µg) were not effective, although one animal of the 0.1 µg-group responded. The animals which had responded to ocular challenge developed respiratory anaphylaxis in response to OA-inhalation (Table 2).

These experiments showed that repeated topical administration of minute amounts of OA (≥ 1 µg) sensitized the IMM/S animals both topically and systemically, but larger doses were needed to elicit the ocular response. After ocular challenge with 5 mg OA, the ELISA anti-OA titers of serum pools of animals immunized with 1 µg, 0.1 µg and 0.01 µg were 2560, 160 and 20, respectively. Heat-stable (but not heat-labile) antibodies to OA with a PCA titer of 80 were demonstrated in a pool of sera from the group immunized with 1 µg OA, but sera from the other two groups were negative (titer ≤ 10).

In a parallel experiment, the minimal dose of OA for sensitizing IMM/S animals (strain 740) to respiratory anaphylaxis was determined. OA was injected subcutaneously five times a week during 2 weeks with the doses indicated, and 2 weeks later the animals were challenged by inhalation. All of 12 animals sensitized with single doses of 1 µg or 10 µg reacted (scores of 2.6–16.0), whereas only two out of eight animals sensitized with doses of 0.1 µg or 0.01 µg reacted (scores ≤ 2.7). This difference is statistically significant ($P < 0.001$, Fisher's exact

test). The threshold doses for sensitization by the conjunctival and the subcutaneous routes thus seem comparable.

Minimal dose for ocular challenge

In this experiment we used IMM/S guinea pigs previously sensitized by topical (conjunctival) instillation of 1 µg or 10 µg doses of OA and challenged with 5 mg doses. After a rest of 1 week, the animals were challenged with the doses shown in Table 3. A dose of 100 µg was not, 500 µg was moderately, and 1000 µg was fully effective for elicitation of ocular anaphylaxis. The difference in the incidence of grade-4 responses to 500 µg and 1000 µg was statistically significant ($P < 0.05$).

Similar experiments have been performed with other groups of sensitized IMM/S animals after rest periods of different duration, with essentially the same results, although 100 µg and 250 µg OA have occasionally elicited weak responses (grades 1–3).

Passive systemic immunization

Three ml of a guinea pig serum pool with an anti-OA PCA-titer of about 1000, essentially IgG₁, was injected intraperitoneally to each of 12 IMM/S (strain 740), 4 IMM/R (strain 203), and 4 IMM/R (strain 201–4b) animals. One week

Table 3

Determination of minimal effective challenge dose for ocular inflammatory response

Guinea pig No.	Date of challenge							
	July 10				July 17			
	Right eye		Left eye		Right eye		Left eye	
	Dose (µg)	Response ^a	Dose (µg)	Response	Dose (µ)	Response	Dose (µg)	Response
142	100	0	500	2	1000	3	500	2
45	100	0	500	3	1000	4	500	4
25	100	0	500	4	1000	4	500	3
84	500	2	100	0	1000	4	500	2
64	500	2	100	0	1000	4	500	3
44	500	1	100	0	1000	4	500	3

Both eyes of the guinea pigs (IMM/S) were previously immunized by topical application of ovalbumin. ^aOcular anaphylaxis evaluated according to an arbitrary scale of 0–5 grades. The 500 µg dose had less grade-4 responses than the 1000 µg dose ($P < 0.05$; Fisher's exact test).

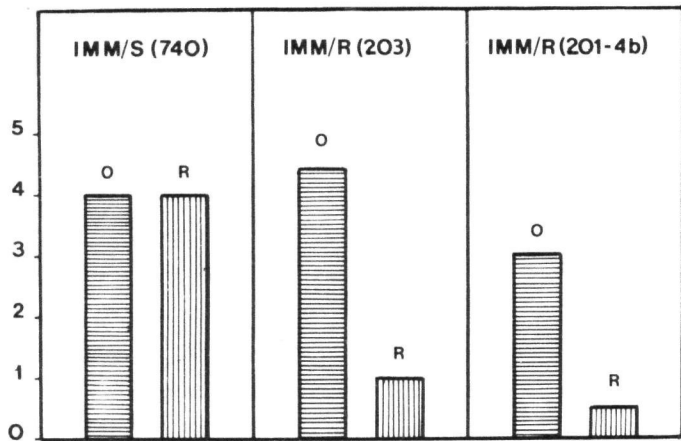


Fig. 3. Responsiveness to ocular (O) and respiratory (R) anaphylaxis in three inbred strains of guinea pigs after passive immunization by intraperitoneal injection of serum containing homocytotropic antibodies to ovalbumin (OA). Twelve IMM/S animals strain 740; 4 IMM/R animals strain 203; and 4 IMM/R animals strain 201-4b were used. The eyes were challenged by instillation of 5 mg OA to the conjunctival sac and the respiratory tract by inhalation of an aerosolized solution of 0.5% OA. The scale indicates the mean of ocular reaction grades and the mean of scores of respiratory anaphylaxis (see Material and Methods).

later the animals were tested for ocular anaphylaxis with 5 mg OA administered topically and later the same day for respiratory anaphylaxis by inhalation. The reactions of the animals are indicated on Fig. 3. The ocular responses of strains 740 (IMM/S) and 203 (IMM/R) were comparable, but 201-4b (IMM/R)-animals were less responsive. The number of grade-4 and grade-5 responders was significantly higher for 740 and 203-animals than for 201-4b-animals ($P < 0.05$). In these passively immunized animals, respiratory anaphylactic responsiveness was strongest for the IMM/S group and weakest for the 201-4b group (Fig. 3).

Passive topical immunization

In these experiments 25 µl guinea pig serum as above was instilled into the conjunctival sacs of naive IMM/S animals. This treatment was given only once (4 animals) or once a day on 10 consecutive days (5 animals). Forty-eight hours after the immunization, the animals were challenged topically with 5 mg OA. No response was observed. A following inhalation of OA had no effect on these animals.

DISCUSSION

Numerous investigators have used guinea pigs as animal models for atopic conjunctivitis. In a long series of careful studies, the team of Dwyer & Darougar (11, 13, 14) immunized the animals by the intradermal route with rabbit serum. On instillation of the allergen into the conjunctival sac, they observed erythema and edema of the conjunctiva as well as excess tearing. Recently, Khatami et al. (18, 19) have shown that the guinea pig conjunctiva can as well be immunized by topical administration of allergen. This has been confirmed in the present study. The milky mucous discharge described in our report was also observed by Khatami et al. (18, 19) but not the diapedesis bleeding (grade-5 response) and the symptoms of general malaise.

The IMM/S and IMM/R animal strains have been bred for high and low responsiveness to respiratory anaphylaxis induced by ovalbumin (OA) inhalations. They differ also in their immune response to synthetic polypeptides (21) and the class-II MHC tissue types which are linked to responsiveness to polypeptides (6, 15) and to some grade associated with the asthma phenotype (9). These data are summarized in Table 4. The IMM/S animals (740, 209), when immunized by inhalation, responded on the first topical challenge of the eye; this was not the case with any of the IMM/R animals, but some of the IMM/R animals (strain 203; line 201-03) could by repeated topical instillation of OA achieve the same degree of responsiveness as the IMM/S animals, whereas other IMM/R strains (201-4b; 201-16) remained refractory (Fig. 2). It should be noted that IMM/S strains and 201-4b are of identical MHC-II tissue type and are responsive to the same polypeptides (Table 4). So our studies seem to urge further elucidation of the biological basis for the connection between tissue type and atopy.

This study allows a provisional division of the inbred guinea pig lines into three immunologically distinct groups:

1. Highly responsive to respiratory and ocular anaphylaxis induced by active and passive immunization with OA (IMM/S: 740).

2. Refractory to respiratory and ocular anaphylaxis induced by active or passive, topical or systemic immunization (IMM/R: 201-4b).
3. Refractory to respiratory and ocular anaphylaxis induced by inhalation, relatively resistant to respiratory anaphylaxis after passive immunization, highly sensitive to ocular anaphylaxis induced by active topical and passive systemic immunization (IMM/R: 203). The latter of these animal groups may be a particularly valuable animal model for the study of human atopic ocular disorders (e.g., vernal keratoconjunctivitis) in patients without other atopic conditions.

Conjunctival instillation may possibly lead to a spread of the antigen to the oropharyngeal cavity and the alimentary tract via the lacrimal canal. This may interfere with immunization (1, 5). We therefore used minute amounts of fluid to dissolve the antigen, hoping to obtain local absorption. In any case, topical application of 1 µg OA proved sufficient for both local and systemic sensitization of the IMM/S animals with responsiveness of the collateral eye, the respiratory tract and the skin. Topical immunization with ≥ 1 µg OA led to IgG-antibody titers of up to 1280. The PCA titer of serum IgG₁ antibodies was relatively low and IgE was not demonstrated. It should be kept in mind that the inflammatory reaction is due to cell-bound antibodies and not to those found in

the circulation. In vernal keratoconjunctivitis patients the concentration of total IgE and of antigen-specific IgE in the tear fluid may far exceed their concentration in the circulation (26-28).

If the 1 µg-dose used for sensitization was actually absorbed by the conjunctival mucosa, this threshold dose was similar to that for effective sensitization of the bronchial tree by the subcutaneous route. In inbred mice, nanogram amounts of subcutaneously injected antigen may elicit an IgE response (31) and in rats immunized with 1 µg ovalbumin, nanogram or even picogram amounts boosted the IgE response (16). In rats the minimal sensitizing dose varies markedly with the inbred strains and may be as large as 100 µg (17).

It is surprising that the minimum dose for effective challenge (500 µg) was 100 times larger than the minimal sensitizing dose . We have not been able to find a similar experimental report. In humans, respiratory anaphylactic attacks may probably be elicited by picogram or nanogram amounts of allergen (7, 22), but our observation may explain certain clinical situations of anaphylactic shock elicited by the first injection of a foreign protein to which the individual had previously been repeatedly exposed albeit by superficial contact only (25). In the animal model, it is quite possible that the effective challenge dose was so large owing to a

Table 4
Characterization of six inbred guinea pig strains

Animal designation	Responsiveness		MHC tissue type ^a		Immunologic response ^b to			Phospho- glucomutase Type ^c
	Respiratory	Ocular	Class I	Class II	PLL	GA	GT	
IMM/S								
209	+	+	B3	Ia2,4	+	+	-	2
740	+	+	B3	Ia2,4	+	+	-	2
IMM/R								
201-4b	-	-	NT	Ia2,4	+	+	-	2
201-16	-	-	B3	Ia1,3	-	-	+	2
203	-	+	B3	Ia1,3	-	-	+	1
201-03	-	+	B1	Ia1,3	-	+	+	2

^a Refs. 8 and 9. In ref. 8 strains 740 and 201-16 are by a mistake indicated as B1. Tissue types of 203 and 201-03 were kindly determined by A. L. de Weck and E. M. Sevach (unpublished). ^b Ref. 21. PLL = homopolymer of poly-L-lysine; GA = copolymer of L-glutamic acid and L-alanine; and GT = copolymer of L-glutamic acid and L-tyrosine. ^c Ref. 29. NT = not tested.

mucosal barrier of non-homocytotropic antibodies (secretory IgA) which may have fixed most of the antigen (30). It should, however, be emphasized that this dose is not high in relation to those used by other authors in experiments with outbred guinea pigs.

Finally, as reported by others (12, 19), "hyposensitization" of the eye could be marked after a single provocation with antigen. The decreased responsiveness could be due to blocking antibodies; activation of T suppressor cells; or to deprivation of the effector cells for mediator substances. The latter possibility is corroborated by the short duration of the hyposensitive stage (Fig. 2) and a pronounced reduction in goblet cells during 24 h after a strong ocular reaction (unpublished, Barishak). Khatami et al. (19) reported that hyposensitization is associated with a sharp reduction in the number of granulated mast cells.

ACKNOWLEDGMENTS

This work was supported by a grant from Else og Svend Madsens Legat. We wish to thank Mrs Pauline Balzer for her expert secretarial help.

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Address:

David Sompolsky, Ph. D., D.V.M.

Rapoport Laboratories for Microbiology

Bar Ilan University

Ramat Gan 52100

Israel

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