

REAGINIC ANTIBODY PRODUCED IN MICE WITH
CONTACT SENSITIVITY

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There has been some speculation that the T-cell-dependent contact hypersensitivity reactions contain elements of both immediate and delayed hypersensitivity. This has been provoked by the finding that the swelling reactions of contact sensitivity can be detected as early as 4 h after challenge (1) and by the demonstration that basophils constitute a significant proportion of the inflammation in contact sensitivity (2). Also lymphocytes have been shown to release a product capable of giving peritoneal exudate cells the ability to passively transfer contact sensitivity (3). The capacity of the sensitized peritoneal cells to passively transfer contact sensitivity was abrogated by treatment with anti-immunoglobulin sera. Askenase (4) has also reported that serum from mice painted with oxazolone can transfer a basophil inflammation when recipient mice were challenged 2 h after serum transfer. This reaction required 24 h after challenge to develop.

More recently the possible contribution of immediate hypersensitivity reactions in contact sensitivity was emphasized by the investigation of Gershon et al. (5) who found that skin sites where contact sensitivity reactions are most easily elicited are particularly rich in mast cells. In addition mice with mast cells depleted of 5-hydroxytryptamine by reserpine could not manifest delayed hypersensitivity reactions although they had sensitized cells capable of passively transferring delayed hypersensitivity to normal recipients. They also showed that serum from mice repeatedly painted with oxazolone could produce a passive cutaneous anaphylaxis reaction in mice if challenged within 2 h of intradermal transfer. This early reaction in mice has been attributed to weak IgG homocytotropic antibody (6). This communication shows that mice can produce reaginic antibody within a week of sensitization with contact sensitizing agents and therefore have a strong homocytotropic antibody capable of arming the basophils in contact sensitivity reactions. The reaginic antibody was unusual in that it was elicited by large doses of antigen without adjuvant and could be boosted by repeated challenge.

Materials and Methods

Mice. 6- to 12-wk-old mice bred at the Clinical Research Centre were used.

Application of Picryl Chloride and Oxazolone. Mice were shaved on the thorax and abdomen and usually painted with either 0.1 ml of 5% picryl chloride (BDH, Poole, Dorset, Great Britain) or 0.1 ml of 3% oxazolone (2-phenyl-4-ethoxymethylene oxazolone) (Sigma Chemical Co., St. Louis, Mo.) on the shaven areas and forepaws. The contact sensitizers were dissolved in ethanol just before application.

Passive Cutaneous Anaphylaxis (PCA). Mouse reagin production was measured by PCA reactions in rats (7, 8). Male albino rats weighing about 200 g were shaved on the abdomen and thorax, and 0.04 ml of sera were inoculated intracutaneously into marked sites, about 16 sites per animal. After 24-h rats were injected intravenously with 2 ml of 0.5% Evans blue dye in phosphate-buffered saline (PBS) containing 15 mg of trinitrophenyl-bovine serum albumin (TNP-BSA). The TNP-BSA was prepared by a slight modification of the method of Rittenberg and Amkraut (9) and had an average of 14 TNP groups per BSA molecule. PCA responses were determined by examining the extravascular extrusion of dye in the reflected skin 45 min after antigen injections. PCA reactions to oxazolone were determined by a similar method using oxazolone-BSA (OX-BSA) prepared as described by Yoshimura and Cinader (10) and conjugated to approximately the same degree as the TNP-BSA.

Each rat was inoculated with a normal serum control and a positive serum obtained from mice immunized with TNP-ovalbumin (TNP-OA) in aluminium hydroxide gel.

Hemagglutination. Serum hemagglutinins were titrated with a Cooke microtitration apparatus (Cooke Laboratory Products Div., Dynatech Laboratories, Inc., Alexandria, Va.). Doubling dilutions were made in PBS containing 1% normal rabbit serum. A 0.5% solution of glutaraldehyde-treated TNP-coated sheep erythrocytes (SRBC) was used for indicator cells. TNP-SRBC were prepared using picryl sulphonic acid (11) and then suspended to 12.5% in PBS containing 0.66% glutaraldehyde, and incubated for 30 min at room temperature before washing. Sera were treated with 0.1 M 2-mercaptoethanol for 30 min at 37°C before assay to determine mercaptoethanol sensitivity.

Before titration sera were absorbed by mixing 0.2 ml serum and 0.1 ml of packed glutaraldehyde-treated SRBC (prepared by incubating 50% SRBC in 1% glutaraldehyde for 30 min at room temperature). This was shown to eliminate the reactivity of normal serum to the indicator cells. Results have been shown as \log_2 of the highest dilution of serum showing definite agglutination after incubating for 1 h at 37°C and overnight at room temperature.

Results

PCA and Agglutinating Antibody. Groups of CBA mice were painted with either 0.5% picryl chloride or 5% picryl chloride on day 0, bled and repainted on day 7 and day 14, and bled on day 21. The serum from each bleed was assayed for agglutinating antibody and for reaginic antibody by PCA in rats. For PCA reactions serum was injected intradermally, 24 h before intravenous challenge with antigen. Two experiments producing similar results have been performed.

The serum from all mice, with one exception, gave PCA reactions when tested at a 1/5 dilution at 1 wk (Table I). Sera on day 14 and 21 gave reactions with blue areas over 2-cm in diameter, whereas reactions from day 7 sera were smaller. An estimate of the reagin titer was obtained by measuring PCA reactions of fivefold dilutions of sera. The reactions had titers of 1/25 to 1/125 which was comparable to the positive control serum produced by immunizing mice with 50 μ g TNP-OA in aluminium hydroxide gel. The titers increased throughout the regime and high antigen dose (5%) gave higher titers than low doses (0.5%). A group of three mice was also painted once with 5% picryl chloride and PCA titers determined after 7, 14, and 21 days. No PCA reactions were found 14 and 21 days after painting.

All mice immunized with high doses of picryl chloride produced mercaptoethanol-sensitive agglutinin (IgM) by day 7, whereas only one of the mice painted with the low dose had a titer (Table II). By day 14 mercaptoethanol-resistant (IgG) titers were found in the high dose group and a mixture of IgM and IgG in the low dose group. The titers did not increase between days 14 and 21.

Heat Sensitivity and Specificity of PCA Activity. 3-wk immune sera from three CBA mice were heated for 30 min at 56°C and the reaction at a dilution of

TABLE I
Reaginic Antibody Produced after Painting with Picryl Chloride

Day	5% Picryl chloride		0.5% Picryl chloride	
	No responding/no immunized (titers)		No responding/no immunized (titers)	
7	2/3 (-, 1/5, 1/5)		3/3 (1/5, 1/5, 1/5)	
14	3/3 (1/5, 1/5, 1/25)		3/3 (1/5, 1/5, 1/25)	
21	3/3 (1/25, 1/25, 1/125)		3/3 (1/25, 1/5, 1/25)	

Mice were painted with 5% or 0.5% picryl chloride on days 0, 7, and 14. Titrations were performed with fivefold dilutions and the last dilution giving a PCA reaction is recorded for each mouse

TABLE II
Agglutinin Production after Painting with Picryl Chloride

Day	5% Picryl chloride		0.5% Picryl chloride	
	-ME	+ME	-ME	+ME
7	4.0 (1.0)	0.3 (0.7)	1.0 (1.7)	0 (0)
14	5.0 (0)	4.7 (0.6)	4.3 (1.5)	2.7 (2.5)
21	4.3 (0.6)	4.3 (0.6)	3.0 (1.0)	3.0 (1.0)

Groups of three mice were painted with 5% or 0.5% picryl chloride on days 0, 7, and 14. Titrations were performed with doubling dilutions and results are the mean (SD) of log₂ dilutions giving positive reactions. ±ME indicates whether titration was performed with or without mercaptoethanol treatment

1/5 compared to the reaction of corresponding untreated sera. Controls gave large reactions, whereas the three heat-treated sera were inactive.

Mice painted with 3% oxazolone every 7 days and bled after 21 days gave positive PCA reactions using OX-BSA as an antigen. The sera did not react with TNP-BSA and conversely sera from mice painted with picryl chloride did not produce reactions with OX-BSA.

Strain Variation. Groups of BALB/c and C57BL/10 mice were painted with 5% picryl chloride on days 0, 7, and 14 and sera taken on days 7, 14, and 21. Unlike the responses of CBA mice no PCA reactions could be detected (at a 1/5 dilution) with serum taken from BALB/c or C57BL/10 mice on day 7 or 14. After 21 days sera from two of five C57BL/10 and three of five BALB/c mice were positive. However, these PCA reactions did not achieve the size of reactions transferred by CBA sera.

Discussion

Mice painted with the contact sensitizing agent picryl chloride produced reaginic antibody detectable within 7 days. The serum factor transferring PCA was characterized as reaginic antibody in that it was produced by specific immunization, could persist in the skin of rats for at least 24 h, and was heat sensitive. In contrast the weak IgG1 homocytotropic antibody of mice is heat resistant, does not transfer to rat skin (even in high doses), and does not persist at the site of inoculation (6-8).

The mice also produced mercaptoethanol-sensitive agglutinins (IgM) after primary skin painting and mercaptoethanol-resistant titers (IgG) after a second painting. High doses of picryl chloride elicited more antibody than low doses. This was found for both reagins and agglutinins. However, the production of reagins and agglutinins differed in that the reagins but not the agglutinins increased after a third painting. It is unusual for experimental animals to

produce increasing amounts of reagin with repeated exposure to large doses of antigen and furthermore reagent production usually requires adjuvant (12, 13). The finding that contact sensitizing agents induce both reaginic antibody and cell-mediated immunity poses the question of why chemically reactive materials elicit immune responses which are normally associated with antigen in adjuvant. An approach to this problem is suggested by the hypothesis that combination of virus and hapten with histocompatibility antigen on the surface of lymphoid cells may be a critical aspect of immunogenicity (14).

Evidence supporting the view that serum-mediated hypersensitivity reactions can have a role in contact sensitivity reactions is as follows: (a) mice painted with contact sensitizing agents produce reaginic antibody, detectable in the serum at least within a week of sensitization; (b) basophils are a significant part of the infiltrate in contact sensitivity lesions (2); (c) mice only readily produce contact sensitivity reactions in regions of skin containing a high proportion of mast cells (5); (d) contact sensitivity reactions are inhibited by compounds depleting animals of vasoactive amines (5). There have been reports showing that vasoactive amines can, under some conditions, reduce cell-mediated responses and could serve as a regulatory mechanism to limit responses (15, 16). However, regardless of the actual role of vasoactive amines in contact sensitivity, the present study shows that mice can produce reagin during contact sensitivity and therefore produce a strong cytotoxic antibody capable of arming the basophils present in contact hypersensitivity reactions.

Summary

Mice produced reaginic antibody within 1 wk of painting with the contact sensitizing agent picryl chloride. The titers, measured by passive cutaneous anaphylaxis in rats, increased after repeated applications of picryl chloride. In contrast, serum agglutinins did not increase after two applications of picryl chloride. Reagin was also elicited by another contact sensitizing agent, oxazolone. Some strain variation of the response to picryl chloride was found, with CBA mice being good responders and BALB/c and C57BL/10 mice being poor responders.

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