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Antigen-specific mast cell degranulation in contact sensitivity to picryl chloride. An early event

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Summary. Mast cells from the peritoneum of mice painted with the contact sensitizing agent picryl chloride degranulate when exposed to antigen (TNP) *in vitro*. Degranulation was consistently demonstrated 4 days after painting which associated with the ability of mice to produce contact sensitivity reactions as measured by ear swelling and radiometric assays. Serum reagin or reagin-producing cells could not be detected until 6 days after painting but TNP-phage neutralizing activity was detected after 2 days. Mast cell degranulation could be elicited by TNP or DNP derivatives indicating the involvement of antibody.

INTRODUCTION

Infiltrates of basophilic leucocytes are found in many delayed-type hypersensitivity reactions including contact hypersensitivity (Dvorak & Dvorak, 1974; Dvorak, Simpson, Bast & Leskowitz, 1971) and in the mouse there is evidence that contact sensitivity responses are dependent on the release of vasoactive amines as well as T cells (Gershon, Askenase & Gershon, 1975; Askenase, 1977). The question of whether the mast cells are specifically armed to respond directly to antigen analogous to their function in immediate hypersensitivity or whether their involvement is indirect is not resolved. Basophils in chronic basophil hypersensitivity (CBH) reactions do not extrude their granules like cells in immediate hypersensitivity reactions but progressively lose the contents indicating a different mechanism of amine release (Dvorak, Mihm & Dvorak, 1976a, b). However, Askenase, Debernardo, Tauben & Kashgarian (1978) have shown that basophils in CBH reactions of guinea-pigs sensitized to proteins in incomplete adjuvant degranulate if appropriate antigen was injected into the lesion. In order to help assess the importance of antigen-induced mast cell degranulation in contact sensitivity, we have studied the sequence of events occurring after skin painting with the sensitizer picryl chloride. It was found that the ability of mice to produce contact sensitivity reactions and the ability of their peritoneal mast cells to degranulate after exposure to antigen *in vitro* appeared between 3 and 4 days after painting even though serum could not mediate passive cutaneous anaphylaxis (PCA) reactions until after 6 days. The degranulation could be elicited by TNP or DNP derivatives indicating the requirement for a B-cell rather than a T-cell product. These results, along with the previous evidence for the requirement

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of vasoactive amine release for contact sensitivity show that mast cell degranulation must be considered as an element in these reactions.

MATERIALS AND METHODS

Mice

Six- to twelve-week old male CBA mice bred at the Clinical Research Centre were used.

Sensitization

Mice were shaved on the thorax and abdomen and a total of 0·1 ml of a 5% solution of picryl chloride (BDH, Poole) was applied to these areas as well as the forepaws.

Detection of contact sensitivity (Ear swelling and radiometric assay)

Mice were injected i.p. with 10^{-7} M 5-fluoro-2-deoxyuridine and after a further 30 min with 2 μCi [^{125}I]-5-iodo-2-deoxyuridine (IUDR). The thickness of the unchallenged ears was determined with an engineers' micrometer. Three hours after IUDR injection one ear of each mouse was challenged on both sides with 5 μl of 1% picryl chloride in a 50/50 (v/v) acetone/di-*n*-butylphthalate solution. After 24 h, the increase in the ear thickness of the challenged ear was measured and then both ears were excised as described by Vadas, Miller, Gamble & Whitelaw (1975), washed three times in ethanol over 2 days and the ^{125}I measured. The measure of sensitivity for the radiometric assay technique is expressed as a ratio of counts in the challenged to the unchallenged ear. The increment in ear swelling is expressed in units of 10^{-3} cm.

Passive cutaneous anaphylaxis (PCA)

Sera were titrated for PCA in rat skin (male Sprague–Dawley) using a 24 h sensitization period (Thomas, Asherson & Watkins, 1976). Titres have been expressed as $\log_2$ of the reciprocal of the last of doubling dilutions producing PCA.

Heterologous adoptive cutaneous anaphylaxis (HACA)

Draining lymph nodes (inguinal, axillary and subscapular) were pressed through a wire grid and the resulting cell suspension was filtered through nylon mesh, washed and suspended at 2×10^7 cells/ml. 0·05 ml of this suspension was injected in three intracutaneous sites in rat skin (male Sprague–Dawley) (10^6 cells/site) and after 24 h, the rats were injected i.v. with 10 mg TNP-bovine serum albumin in 2 ml of saline containing 0·5% Evans blue dye. After 45 min, the diameter of the area of extravasated dye was determined by two measurements taken at right angles. The arithmetic mean of diameters of the three sites was taken as measure of HACA. This general method has been described by Kind & Macedo-Sobrinho (1973). Using sensitization with oxazolone-conjugated BSA it was shown that the correct antigen was required. Lymph node cells incubated with immune serum *in vitro* for 3 h could not mediate HACA reactions.

Mast cell degranulation

Mast cells were collected in siliconized glassware by washing the peritoneum of mice with 5 ml 199 medium with 1% heparin. The peritoneal cells were washed with centrifugation (300 ***g***, 10 min) and incubated with 500 μg/ml antigen (TNP-BSA, oxazolone-BSA, BSA or DNP-BSA) for 20 min at 37 °. A drop of the cells was placed on a slide stained with 3% neutral red solution in absolute alcohol. The percentage of degranulated mast cells was determined after counting about 200 cells. The % specific degranulation has been scored as % degranulated without antigen $-$% degranulated with antigen. During experiments, peritoneal mast cells from ten normal mice were tested for degranulation. Only one of these mice showed any specific degranulation. The technique has been described in detail (Schwartz & Vardinon, 1966).

Phage-inactivating antibody

Techniques similar to those used by Jormalainen & Mäkelä (1971) were used. TNP-coupled phage was prepared by incubating 13×10^9 plaque-forming units (PFU) of T2 phage in 1 ml of 0·1 mg/ml picryl sulphonic acid (BDH Poole) in borate-buffered saline pH 8·4 at room temperature for 1 h. 0·1 ml of glycine was then added and the mixture dialysed. This procedure retained 24·5% of the PFU activity. Phage was purchased from Miles Laboratories (Slough). To assay PFU, 20 μl of a sample diluted in PBS, 2% agamma horse serum was added to 2 ml of sloppy agar (0·8% Bacto agar, 0·1% Difco tryptose and 0·5% sodium chloride) kept at 45°. Two drops of an overnight broth of *E. coli* B was added and the mixture overlayed on a nutrient agar plate. Plaques were measured after 24 h at 37°. The antibody titrations reported here were performed by incubating 10 μl serum and 10 μl phage to 80 μl PBS-2% agamma horse

serum and incubating for 4 h at 37° before assaying for PFU.

RESULTS

Development of contact sensitivity

Mice were painted with picryl chloride and contact sensitivity measured at daily intervals. When measured by ear swellings, the reactions were not elicited after 2 days but a peak of reactivity was found if they were challenged after 3 days (Fig. 1). In the four experiments shown in Fig. 1, the swelling reactions elicited on day 4 and 5 were smaller than those found after challenge on day 3 but the response is apparently biphasic in that responses elicited after day 5 can be as large or larger than day 3 responses (Table 1). Sensitivity was also measured by the radiometric cell arrival test in which mice were injected 3 h before challenge with ^{125}I-IUDR to label cells before eliciting sensitivity. The mice were then challenged on an ear and the radioactivity in the ears was measured after 24 h. Sensitivity was calculated as an index of the radioactivity in the challenged ear to that in the unchallenged ear. Measured this way sensitivity was detectable after 3 days but was maximal when challenged on day 4 (Fig. 2).

Table 1. Biphasic contact sensitivity response measured by ear swelling

Days after sensitization	Ear swelling (increment 10^{-3} cm)	IUDR-cell arrival ratio
0	7·2 (2·2)	2·0 (0·3)
3	21·9 (4·5)	4·6 (0·4)
5	13·8 (1·7)	3·2 (0·8)
9	25·3 (5·7)	4·1 (0·7)

Mice were painted with picryl chloride and after the intervals shown groups were challenged on an ear and sensitivity measured by the IUDR-cell arrival index and ear thickness. Results show mean (SD) of groups of four mice.

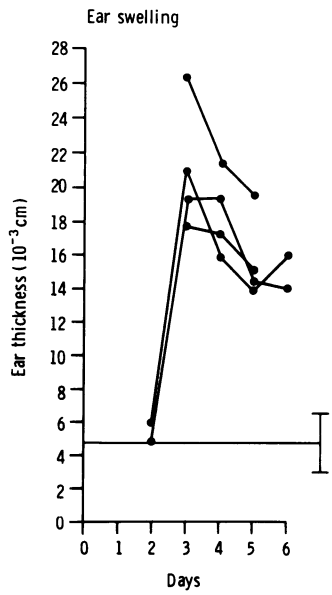

Figure 1. Contact sensitivity measured by ear swelling. Mice were painted with picryl chloride on day 0 and challenged on the days shown. The increase in ear thickness was measured after 1 day. Results show means in groups of four mice for four experiments (see Tables 1 and 3 for representative standard deviations).

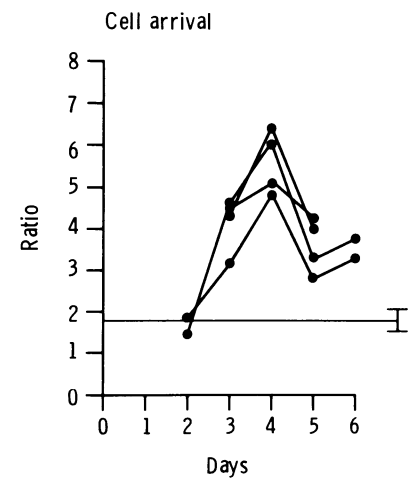

Figure 2. Contact sensitivity measured by radiometric test (IUDR-cell arrival). The ratio of radioactivity in challenged: unchallenged ears of mice painted on day 0 and challenged on the day shown is given for four experiments. Each point is the mean of four mice (see Table 1 for representative standard deviations).

Antibody titrations

Sera from sensitized mice were tested for anti-TNP reagin by 24 h PCA in rat skin and also for the development of TNP-T2 phage neutralizing activity. Anti-TNP-T2 was detectable after 2 days and increased thereafter to 6 days (Fig. 3). Sera from ten untreated mice did not neutralize the phage and sera taken on days 2, 3 and 6 of the experiment shown in Fig. 3 did not inhibit unmodified T2. Reagin titres as measured by passive cutaneous anaphylaxis (PCA) in rat skin with a 24 h sensitization period are shown in Fig. 4. This was not detectable until 6 days after painting. Production of reagin by lymph node cells was measured by the heterologous adoptive cutaneous anaphylaxis (HACA) test in rat skin using 10^6 cells/injection site. Consistent reactions were not found until day 6 (Fig. 4).

Mast cell degranulation

Mast cells from peritoneal washings were exposed to TNP-BSA for 20 min and examined for degranulation. Preliminary tests showed that cells from sensitized mice did not degranulate significantly after exposure to BSA or oxazolone-BSA but did degranulate after exposure to TNP-BSA. The ability of mast cells taken at daily intervals to degranulate after exposure to TNP-BSA is shown in Fig. 5. Consistent degranulation was found after 4 days but could be detected earlier in some mice. During the experiments, cells from ten normal mice were also tested for degranula-

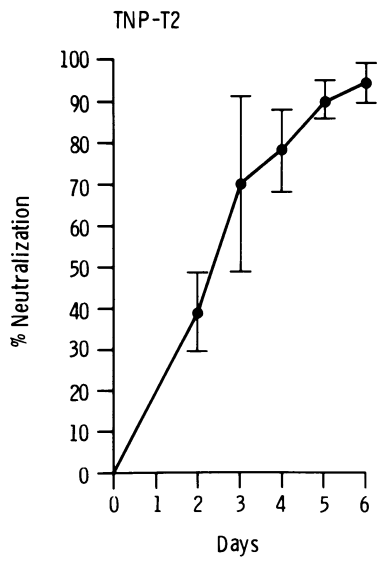

Figure 3. Serum TNP-T2 neutralizing activity. Results show the mean (SD) of the percentage neutralization from groups of four mice painted with picryl chloride on day 0. Normal serum did not neutralize (day 0).

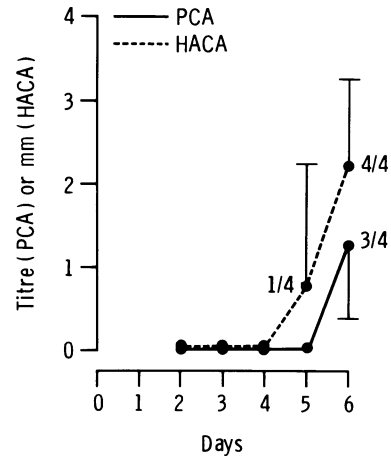

Figure 4. Anti-TNP serum reagin and heterologous adoptive cutaneous anaphylaxis reactions (HACA). Serum reagin was determined by PCA ($\log_2$ of dilution of serum inducing a reaction) and HACA shows the diameter of HACA reactions produced by 10^6 lymph node cells. Each result in the mean (SD) of groups of four mice. The fractions show responder/non responder.

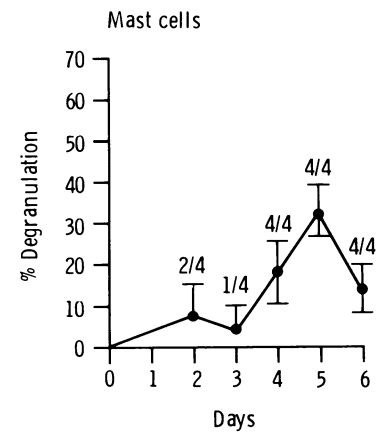

Figure 5. Mast cell degranulation. Peritoneal washings from mice painted on day 0 were incubated with TNP-BSA and the degranulation of mast cells examined. Results show mean (SD) of specific degranulation (degranulation with antigen minus degranulation without) of cells from groups of four mice. The fraction gives number of washings showing greater than 5% specific degranulation. Specific degranulation was found in only 1/10 normal mice.

Table 2. TNP-DNP cross-reactivity of mast cell degranulation

Mouse No.	TNP	DNP
1	30	30
2	12	15
3	5	0

Peritoneal cells taken from mice 4 days after painting with picryl chloride were incubated with TNP-BSA or DNP-BSA. Both reagents caused significant degranulation in 2/3 mice (> 5%).

tion after exposure to TNP-BSA. One sample showed 10% degranulation but all others were negative.

DNP-TNP specificity

Mast cells in peritoneal washings taken 4 days after skin painting degranulated after exposure to TNP-BSA or DNP-BSA (Table 2). In contrast, the ability of DNFB to elicit contact sensitivity in mice activity sensitized to picryl chloride or in mice injected with cells from mice painted with picryl chloride was negligible (Table 3).

DISCUSSION

Mast cells from the peritoneal cavity of mice painted with picryl chloride degranulate when exposed to antigen (TNP) *in vitro*. This activity can be consistently found 4 days after painting and associates with ability of mice to produce contact sensitivity reactions which can be found when mice are challenged 3 days after painting and the reactions read on day 4. The degranulation is probably mediated by antibody because it could be elicited by TNP or DNP conjugates. Cell-mediated contact sensitivity responses did not show this cross-reactivity (Table 3) and similar fine specificity has been found for a DNP-specific suppressor factor (Moorhead, 1977). In contrast, PCA reactions can be elicited with TNP or DNP conjugates regardless of whether picryl chloride or DNFB was used to produce the reagin (data not shown).

Serum antibody as measured by neutralization of TNP-phage was found 2 days after painting, but reagin or cells producing reagin measured by PCA in rat rat skin, was not found until 6 days after painting. Therefore if reagin is involved it must be produced in small quantities and concentrated on mast cells. The antibody detected by phage neutralization could be IgM as judged by the early appearance of anti-TNP haemolytic plaque-forming cells (Thomas, Watkins, Jouhal & Asherson, 1978). The possibility that the neutralizing activity is not classical antibody has, however, not been excluded.

Contact sensitivity measured by ear swelling was at a peak when mice were challenged on day 3 and sensitivity measured on day 4. This response measured by the arrival of IUDR-labelled cells was maximal a day later. Because the cells were actually labelled in the sensitized mice this difference may represent differences in the IUDR incorporation of cells travelling to lymph nodes rather than the number of cells in a reaction. However, if it does represent differences in the number of cells arriving then the arrival of extra cells on day 4 decreased the size of the reactions, as measured by swelling.

Table 3. TNP-DNP cross-reactivity of contact sensitivity

	Challenge PIC	Challenge DNFB
Active sensitization (painting with picryl chloride)		
Paint (PIC)	25·8 (2·6)	2·5 (0·8)
No paint	5·7 (0·9)	1·5 (0·6)
Passive transfer (PIC cells)		
cells	5·6 (1·1)	1·0 (1·3)
no cells	2·9 (1·0)	0·3 (0·3)

Mice were actively sensitized by painting picryl chloride and challenging after 7 days. For passive transfer lymph node and spleen cells were taken from mice 3 days after painting and recipients challenged immediately after transfer. The challenge dose of DNFB (0·4%) produced sensitivity in mice painted with DNFB (not shown). Results show mean (SD).

It should also be noted that the contact sensitivity response is at least biphasic and although sensitivity declined by day 3–5, responses measured later are larger or as large as day 3 responses. Perhaps this is related to the fact that earlier contact sensitivity responses can be transferred by cells from the lymph nodes and spleen whereas later responses can be transferred by cells from the bone marrow or cells found in peritoneal exudates (Asherson & Zembala, 1973), i.e. different types of cells mediate early and late responses.

An involvement of mast cells as well as T cells for contact sensitivity in the mouse has been indicated because contact sensitivity reactions can be elicited only in sites rich with mast cells (mice have no basophils) and inhibitors of vasoactive amines inhibit sensitivity (Gershon *et al.* 1975; Askenase, 1977). Frank degranulation involving extrusion of granules does not occur in contact sensitivity but there does appear to be a steady loss of the contents of the granules (Dvorak *et al,* 1976a, b). Degranulation of basophils in CBH reactions can, however, be produced by injecting antigen into the lesions (Askenase *et al,* 1978). In this context, it should be noted that degranulation would be dependent on the amount of homocytotropic antibody (or other factors) present, the degree of infiltrate (because of feedback mechanism) and also the antigen presentation.

The effects of vasoactive amines on the reactions, and the reason for their requirement, could involve interaction of several factors which include permeability changes, inhibitory effect of histamine on T effector cells (Plaut, Lichtenstein & Henney, 1976; Rocklin, 1976), the binding to suppressor T cells (Shearer, Melmon, Weinstein & Sela, 1972) as well as ability of histamine to feed back on mast cells (Lichtenstein & Gillespie, 1973). The complexity of the outcome of these factors is illustrated by the findings that adding histamine to delayed hypersensitivity reactions has been shown to be inhibitory (Rocklin, 1976) while blocking H-2 receptors is also inhibitory (Askenase, 1977); whereas IgE antibody can both augment or inhibit delayed hypersensitivity reactions (Pearson & Taylor, 1977).

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