

Increase in skin mast cells following chronic house dust mite exposure

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SUMMARY

Application of inhalant allergens in high concentration to the mildly abraded skin of sensitive patients with atopic dermatitis gave rise to eczematous skin responses at 48 h. These lesions, infiltrated by basophils, eosinophils and mononuclear cells, are examples of cutaneous basophil hypersensitivity. Repeated application of allergen induced an increase in skin mast cells by 6 days, the mast cell hyperplasia replacing the earlier basophil infiltration. No electron microscopic evidence of mast cell heterogeneity among the recruited cells was found.

The naturally occurring lesions of chronic atopic dermatitis are infiltrated by mononuclear cells and show increased numbers of mast cells in the dermis (Mihm *et al.*, 1976). The lymphocytes present have both T helper and T suppressor phenotypes, suggesting a form of delayed hypersensitivity reaction (Leung *et al.*, 1983). The cell infiltrate usually does not include basophils or eosinophils, so that it is not typical of cutaneous basophil hypersensitivity, a form of delayed hypersensitivity reaction, which is characterized by these cells (Askenase 1977; Mitchell & Askenase, 1983). In renal allograft rejection (Colvin & Dvorak, 1974) and allergic contact dermatitis (Dvorak, Mihm & Dvorak, 1976) the early presence of basophils may be followed by an increase in mast cells and it remains unclear whether this is due to cell recruitment or to hyperplasia. It has been suggested that this same sequence could occur in the lesions of atopic dermatitis (Mihm *et al.*, 1976).

Patients with atopic dermatitis often have high levels of IgE antibody and positive immediate hypersensitivity to common inhalant allergens (Chapman *et al.*, 1985). We have previously demonstrated that these same inhalant allergens applied to the skin for 48 h can induce a delayed eczematous response with microscopic features of cutaneous basophil hypersensitivity (Mitchell *et al.*, 1982; Mitchell *et al.* 1984). In these lesions mast cell numbers were not increased. In

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the present study, we have demonstrated that repeated administration of allergen at high concentration to the same site results in the development of eczematous lesions in which a basophil infiltrate is replaced by a mast cell infiltrate. On electron microscopy the recruited mast cells were morphologically the same as the mast cells normally resident in the skin.

METHODS

Patients

Five patients who had suffered from atopic dermatitis since infancy were studied while in remission, using a modified patch test technique. All had suffered from severe and extensive skin inflammation and had positive immediate skin test reactions to *Dermatophagoides pteronyssinus* extract. Four also had bronchial asthma. The mean age was 25 years (range 20–30) and one patient was female. The experiments reported here were approved by the Northwick Park Hospital Ethical Committee.

Allergens

Antigen P₁ was purified from an extract of *D. pteronyssinus* culture as previously described (Chapman & Platts-Mills, 1980). Ragweed allergen was obtained from Center Laboratories (North Carolina, U.S.A.). For patch tests, a 2 × 2 cm area of skin was gently abraded with a scalpel blade, removing the upper layers of the epidermis. The sites included the flexor aspect of the forearm, the outer aspect of the upper arm, and the back, medial to the scapula. Control patch test sites were always placed in a comparable position. Allergen diluted to 10 µg/ml (or less) in normal saline (0.5 ml) was applied to a pad of sterile gauze, occluded with plastic and retained in position using hypoallergenic tape. After 2 days the sites were inspected, visually graded and either biopsied or had the same quantity of allergen applied for a further 2 days. Allergen application was repeated at 2-day intervals up to 8 days.

Biopsies

Biopsies were taken after 2, 6 and 10 days, under local anaesthetic using a 4-mm disposable skin biopsy punch. Biopsy specimens were fixed in Karnovsky's solution for 24 h, washed in four changes of 0.1M sodium cacodylate buffer, pH 7.4 at 4°C, and dehydrated in varying concentrations of alcohol. Subsequently, these specimens were embedded in glycol methacrylate and 1–2 µm sections were cut and stained with haematoxylin and eosin (H&E), a modified Giemsa technique and with Azure A. All sections were examined under a × 100 oil immersion lens, and infiltrating cells were identified and systematically counted over the whole section. For electron microscopy, specimens fixed in Karnovsky's solution for 24 h were post-fixed in osmium tetroxide, dehydrated through graded acetone and embedded in Spurr resin. Sections (1 µm) were stained with toluidine blue, and thin sections were collected on copper grids, stained with uranyl acetate and Reynold's lead citrate and viewed in either a Philips 300, or JEOL 1200 electron microscope.

Morphometric measurements of the section widths and of the percentage of scrolled areas in the mast cell granules were performed using a Joyce-Loebl Magiscan I Image Analysis System. The widths of the light microscope sections were measured by drawing the maximum width of the H&E section onto its image on the Magiscan screen with a light pen and allowing the computer to measure the length of the line. To measure the percentage area of mast cell granules occupied by scrolls, very pale EM prints were prepared and the scrolled areas were hand shaded with black ink in order to give enough contrast for the Magiscan to differentiate the scrolled from the unscrolled areas.

RESULTS

All patients studied were known to have a high IgE level (geometric mean 5617 IU/ml; range 2900–9900) and specific IgE antibody to antigen P₁ (geometric mean 203 BA units/ml; range 19–768) (Chapman & Platts-Mills, 1980) and had been found to give an eczematous patch test response to antigen P₁, the major house dust mite antigen. As had been shown previously, these reactions were seen at day 2 at sites where antigen P₁ (10 µg/ml) was applied but not at control sites where saline alone was applied. The positive lesions were characterized by the presence of erythema, papules, induration and exudation. In four patients the re-application of antigen P₁ every 2 days gave rise to a gradual change in the character of the lesions with less erythema, but

TABLE 1. Numbers of infiltrating cells in patch test sites*

Patient†	Antigen‡	Day§	Basophils	Mast cells	Eosinophils	Mononuclear cells
1	Control	2	7	70	15	239
	P ₁	2	66	70	972	1445
	P ₁	6	33	107	276	772
	P ₁	10	7	149	130	600
2	Control	2	n.d.	n.d.	n.d.	n.d.
	P ₁	2	17	88	559	854
	P ₁	6	3	103	210	506
	P ₁	10	5	145	117	410
3	Control	2	6	111	1	634
	P ₁	2	38	129	96	988
	P ₁	6	36	139	219	1053
	P ₁	10	13	115	36	561
4	Control	2	1	49	7	206
	P ₁	2	26	49	310	630
	P ₁	6	34	138	837	529
	P ₁	10	18	144	65	341
5¶	Control	2	0	24	1	141
	P ₁	1	33	49	59	606
	P ₁	2	52	39	376	482
	P ₁	3	40	48	60	1211
	P ₁	7	17	46	116	374
1†	Ragweed	2	1	56	17	415
	P ₁ 0.1 µg	2	5	62	56	476
	P ₁ 1 µg	2	17	55	82	523
	P ₁ 10 µg	2	26	65	155	781

* Histological sections examined were comparable in width. All infiltrating cells and resident mast cells in the dermis were counted.

† Patients 1–4 had repeated antigen P₁ application. In patient 1 a further study was done on day 2 on a separate occasion, when antigen P₁ was applied at different concentrations for 48 hours prior to biopsy.

‡ Antigen P₁ was used at 10 µg/ml unless otherwise stated. Ragweed allergen was used at 10 µg/ml in patient 1.

§ Number of days between first application of antigen and subsequent biopsy.

¶ Patient 5 had allergen applied to different sites at day 0 and left *in situ* for varying time periods prior to biopsy.

n.d., Not determined.

further induration and crusting becoming apparent. By day 10 the centre of the lesions had become rather pale and was surrounded by erythema and scaling. In the case of one patient, antigen P₁ was applied on a single occasion and the lesions appeared maximum at day 3 and had almost disappeared by day 7 with little erythema, few papules and no oedema or crusting. All subjects reported increased systemic itching associated with repeated application of patches over 10 days, particularly noticeable at days 2 and 4.

Biopsies of parallel allergen patches were taken at 2, 6 and 10 days. The cellular infiltrate present in all subjects at day 2 was similar to that previously reported, and included basophils, eosinophils, mononuclear cells and neutrophils (Table 1). The number of these cells was maximal between day 2 and day 6 (Table 1; Fig. 1). Mast cells, however, behaved differently. Over the 10-day period of repeated application, a greater than 50% expansion in mast cell numbers developed (Fig. 1). As is evident from Table 1, this pattern was seen in three out of four subjects, whereas in the fourth a minimal increase in numbers was associated with a particularly large number of skin mast cells in the original and the control biopsies. In contrast, a single application of allergen to each site in one patient produced an increase in basophils, eosinophils and mononuclear cells but did not induce any increase in mast cells over 7 days (Table 1).

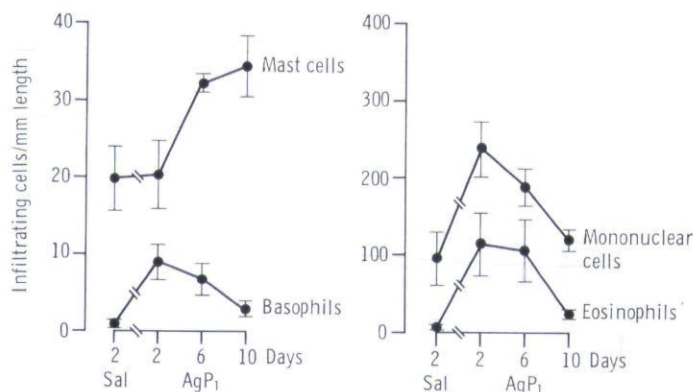


FIGURE 1. Number of infiltrating cells per unit (mm) length in patch test sites repeatedly exposed to antigen P₁ (days 2, 6 and 10) and saline (day 2). Results expressed as mean $\pm$ s.e.

TABLE 2. House dust mite antigen P₁ levels in patients' homes*

Patient	Antigen P ₁ μ g/g fine dust		
	Mattress	Bedclothes	Bedroom floor
1	64.1	62.6	88.0
2	13.5	38.5	15.6
3	164.2	41.8	383.8
4	n.d.	22.8	28.6
5	94.3	200.0	561.6

*Antigen P₁ measured by vacuuming particular sites, collecting the dust samples, extracting and analysing using a double antibody inhibition radioimmunoassay.
n.d., Not determined.

Recruitment of other cell types in this experiment was comparable to that seen with repeated patch testing.

The concentration of antigen P_1 applied was large in comparison with that required to elicit immediate hypersensitivity. However, these patients were exposed to high levels of antigen P_1 in their homes (Table 2). A dose response on one patient showed a progressive increase in basophil and eosinophil infiltration from 0.1–10 $\mu\text{g/ml}$ (Table 1). In addition, as was also reported previously, an antigen to which the patient had not been exposed, i.e. Ragweed, failed to induce significant cell infiltration (patient 1; Table 1).

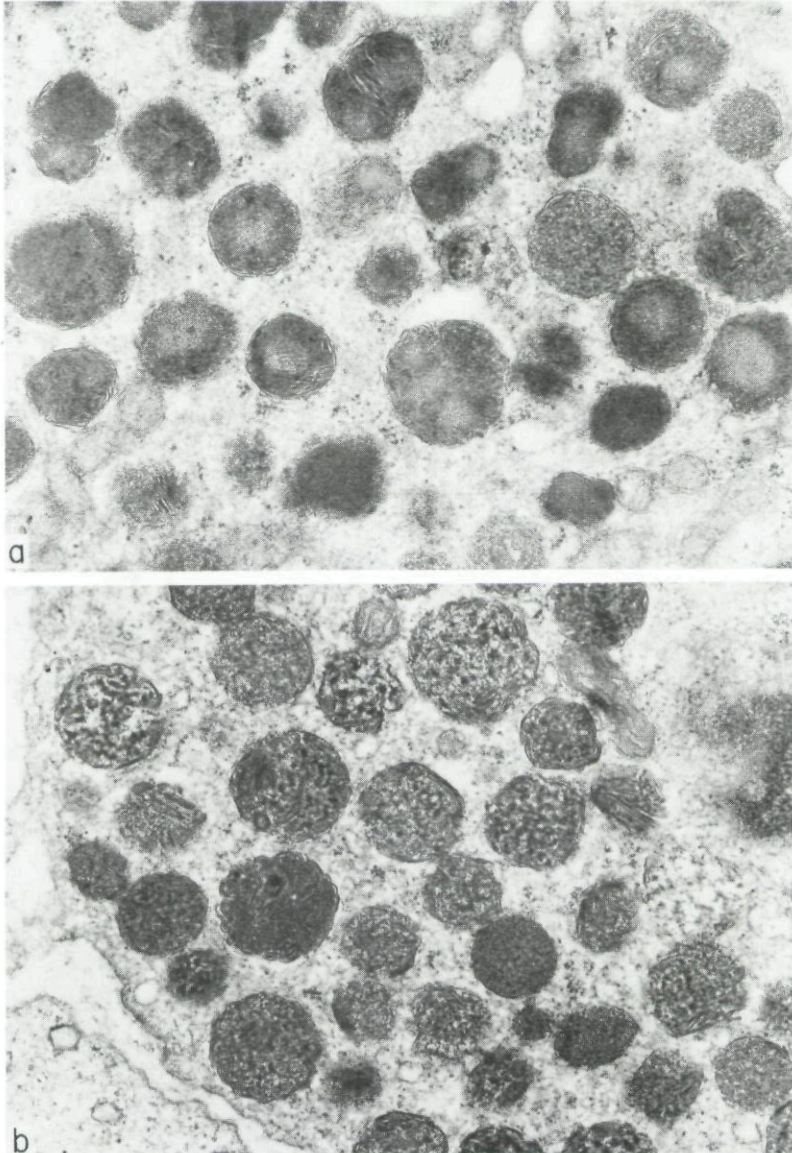


FIGURE 2. (a) Typical mast cell granules at day 2 from saline patch test site (Original $\times 34,020$). (b) Mast cell granules from antigen P_1 site biopsied after 10 days repeated application. The granules show a variegated texture and prominent scroll structures (Original $\times 34,020$).

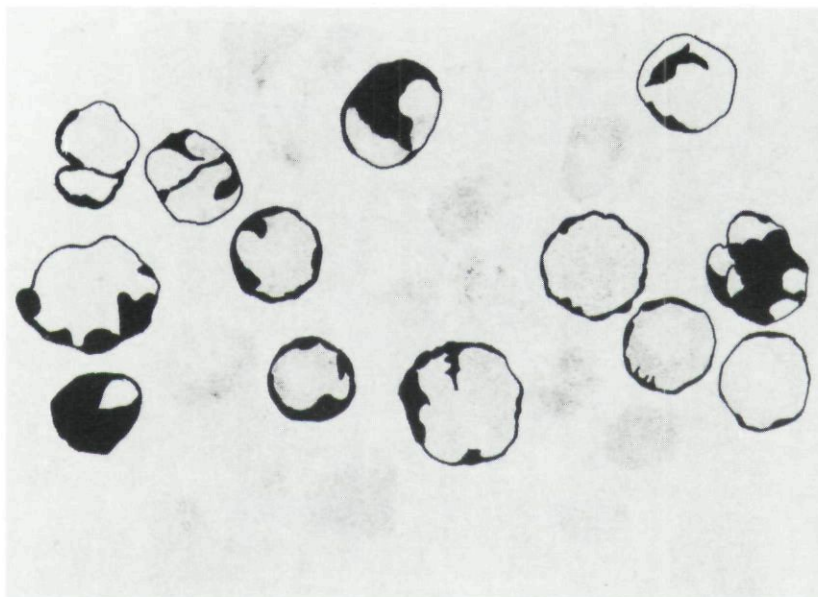


FIGURE 2. (c) Pale print of electron micrograph has been hand shaded with black ink to define areas containing scroll structures for measurement by the magiscan (Original $\times 34,020$).

An electron microscopic analysis of mast cells in the lesions biopsied at different time points was performed. It was not possible to identify any definite morphological differences in the mast cells present in antigen P_1 or saline test sites at day 2, day 6 and day 10 (Fig 2a and b). However as some mast cell granules in the day 10 lesions did appear to have more prominent scroll structures, a large number of the granules (117) were analysed on the Magiscan Image Analysis System. There was no difference between the percentage area of granules occupied by scrolls in day 2 and day 10 lesions (42% versus 39%) (Fig. 2c).

DISCUSSION

Detailed study of the morphology of inhalant allergen induced patch test reactions has shown that early basophil infiltration is subsequently replaced by an increase in mast cells. In previous human studies of contact dermatitis, similar results have been obtained, with basophil infiltration being replaced by inflammation in which mast cells become a feature (Dvorak *et al.*, 1976). We have found that to induce this change in patients with atopic dermatitis it was necessary to apply allergen repeatedly to the same skin site for 1 week or longer. Such repeated application closely mimics the natural exposure to house dust mite in the home where patients' skin is in direct contact with high concentrations of allergen for prolonged periods each day or night (Tovey, Chapman & Platts-Mills, 1981). The failure of a single application of allergen to induce mast cell changes further supports the need for chronic exposure. The concentration of allergen used in our tests was in keeping with both the home exposure levels, and the antigen concentrations required to induce cutaneous basophil hypersensitivity in experimental guinea-pigs (Askenase, 1977). We have demonstrated that low concentrations of allergen give rise to a

lesser degree of cellular infiltration. It is of interest that the chronic lesions of atopic dermatitis are not infiltrated by basophils and eosinophils, but do have increased numbers of mast cells. Again in the patch test lesions, eosinophils were decreasing in numbers at a time when mast cells were increasing. Thus, repeated application of allergen to the skin results in lesions that macroscopically and in terms of the microscopic cellular infiltrate, resemble chronic lichenified eczema.

It has now become clear that tissue mast cells are probably not a homogeneous population, since differences based upon fixation and staining, and functional characteristics have been identified in rodents and in man (Strobel, Miller & Ferguson, 1981; Metcalfe, 1983). We considered the possibility that the increase in mast cells produced in the skin would represent infiltration by a subpopulation of these cells. However, detailed ultrastructural examination failed to show any striking differences in mast cells present on day 2 and day 10. Our observations (E.B. Mitchell *et al.*, unpublished) on human skin and intestinal mast cell granules suggest that there is more active granular scrolling in mucosal than in connective tissue mast cells, although this may represent differences in the degranulation cycle rather than heterogeneity. We carefully analysed the mast cell granules in the patch test biopsies for the percentage area of the granule occupied by scroll-like structures, but found no significant differences. We conclude that the mast cell expansion that occurs does not involve cells that differ structurally. This, of course, does not rule out a functional heterogeneity.

Basophil recruitment in experimental animals is a regulated phenomenon, predominantly associated with T-cell function (Askenase, 1977; Mitchell & Askenase, 1982). In man it is apparent that basophils will exhibit chemotaxis to lymphocyte products and activated complement components (Lett-Brown, Boetecher & Leonard, 1976). We have demonstrated that patients with house dust mite allergic disease have sensitized T-cells and that allergen exposure *in vitro* can induce those sensitized lymphocytes to produce a basophil chemotactic activity, which appears to be due to a factor with a molecular weight of approximately 20 000 daltons (Mitchell *et al.*, 1984). The expansion in mast cell numbers that develops with chronic antigen exposure may be due to cell differentiation or replication (Galli & Dvorak, 1984). In experimental animals a particular subset of mast cells has been shown to differentiate under T lymphocyte control (Davidson *et al.*, 1983). The basophils and mast cells that are recruited or that differentiate at a chronic allergic inflammatory site presumably have an effector function. This may be one of alteration in vascular tone or permeability and may be dependent upon the production of mediators via an IgE dependent pathway (Schleimer *et al.*, 1983). However, new evidence in mice suggests that the immune response controlling mast cells can also involve T-cells (Askenase *et al.*, 1983). An antigen specific T-cell factor that results in mast cell mediator release has been demonstrated. Furthermore, a number of inflammatory mediators such as interleukin-2 and gamma interferon are known to alter mast cell mediator release (Lett Brown *et al.*, 1981). It is likely therefore that the cellular inflammation that occurs following the application of allergen to the skin involves T-cells as well as IgE antibody and that T-cell regulation including the switch from basophil to mast cell infiltration is in progress. It is possible that mediators released from basophils and mast cells, e.g. histamine, have a regulatory role as well as being vasoactive and may influence the inflammatory response that develops in the skin (Rocklin *et al.*, 1978). Of interest is the observation that, in spite of continued allergen exposure, the increased numbers of mast cells present do not appear to be degranulated or to continue active recruitment of eosinophils as they had done earlier following allergen application. Therefore, the exact role of this cell type in chronic inflammation remains unclear.

From our observations we would suggest that the immunological process in atopic dermatitis

can be explained by cellular immune mechanisms induced by allergen exposure. These mechanisms involve a form of delayed hypersensitivity response and presumably in the early stages, overlap with the mast-cell mediated late phase responses (Solley *et al.*, 1976). It is important to remember that there are many different changes in the skin of these patients, including biochemical and neurovascular changes. It is not at all clear whether these changes are primary or could be secondary to prolonged inflammation. If allergens can be implicated in the inflammation of atopic dermatitis, attempts at avoidance are necessary and in the case of the house dust mite it is now clear that marked reductions in allergen levels throughout the home can be achieved (Mitchell *et al.*, 1985).

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