

Cutaneous Basophil Hypersensitivity to Inhalant Allergens in Atopic Dermatitis Patients: Elicitation of Delayed Responses Containing Basophils Following Local Transfer of Immune Serum but Not IgE Antibody

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Inhalant allergens applied to the skin of sensitive atopic dermatitis patients by means of a modified patch test technique, induce acute eczematous lesions. These lesions contain basophils, eosinophils, mononuclear cells, and neutrophils and represent an example of human cutaneous basophil hypersensitivity. The role of IgE antibody in this eczematous reaction was studied by systemic and local passive transfer experiments. Plasma with high IgE antibody when infused into patients with hypogammaglobulinemia as part of their replacement treatment resulted, post infusion, in cutaneous mast cell and blood basophil sensitization as measured by quantitative skin testing and leukocyte histamine release. Subsequent patch tests on these patients using the house dust mite antigen, antigen P₁, produced macroscopic erythematous responses containing mononuclear cells, and eosinophils but not basophils. Local transfer of atopic dermatitis serum with high IgE antibody produced weak macroscopic responses and in these lesions mononuclear cells and both basophils and eosinophils were present. The serum activity which allowed transfer of basophil and eosinophil recruitment was heat labile. Specifically purified antibody to the mite antigen P₁ (containing IgE and IgG antibody), when transferred, allowed eosinophil but not basophil recruitment to patch test sites. These results suggest that while the allergen-induced patch test response may involve IgE antibodies, as well as the cells normally involved in delayed responses, another serum activity is also involved.

The immune mechanisms involved in the pathogenesis of atopic dermatitis are unclear. The cellular infiltrate present in the chronic lichenified lesions of this disease suggests a form of delayed hypersensitivity (DH) reaction [1]. On the other hand, individuals with this disease frequently have very high levels of IgE antibody and, when skin tested, show wheal-and-flare responses in 10 min to multiple different allergens [2,3]. Proof that the allergens that give rise to immediate responses

are a cause of the skin lesions or of the cellular infiltrate of this disease is lacking. Recently, however, we have reported that aqueous allergen extract applied as a patch test to the mildly abraded skin of patients with atopic dermatitis can induce an eczematous reaction in 48 h [4]. These lesions are infiltrated with basophils and eosinophils as well as mononuclear cells and neutrophils, and appear to represent a form of cutaneous basophil hypersensitivity (CBH) in humans [5,6]. Dvorak and Mihm have reported that basophils and sometimes eosinophils are present in induced contact hypersensitivity lesions in humans [7]. Similarly, basophils have been reported to be part of the indurated late-phase reactions that sometimes follow immediate hypersensitivity reactions [8-11]. It now appears that CBH may be a common form of human cellular immunity [12,13].

In guinea pigs, CBH is regarded as a form of delayed sensitivity that is distinct from classical DH. Basophils are very rare in the cellular infiltrate of classical DH. The ability to demonstrate CBH is lymphocyte-dependent [14] and responsiveness can be adoptively transferred with T cells from immune animals [15,16]. Under some circumstances, it is also possible to transfer CBH reactivity with intravenous injections of immune serum, and the active constituent of the serum appears to be IgG₁ antibodies [17]. This class of guinea pig antibodies can passively transfer the ability to elicit cutaneous anaphylaxis locally. By contrast, it has not proved possible to transfer CBH responsiveness with local injections of IgG₁ antibodies. This suggests that the elicitation of CBH requires more than simple binding of IgG₁ antibodies to mast cells. Further evidence that serum factors regulate basophil accumulation comes from animals immunized with protein emulsified with complete Freund's adjuvant. These animals normally demonstrate DH, but when their T cells are transferred, the lesions elicited in the recipients represent CBH [16]. However, if both T cells and serum from the immune animals are transferred, basophil recruitment is suppressed [18]. Thus, the recruitment of basophils into the skin of actively immunized animals is under complex regulation.

Since both CBH in guinea pigs and the late-phase reaction in humans can be passively transferred with serum, we wondered whether the eczematous delayed response elicited in allergic humans could be transferred. We have carried out systemic transfer of IgE-rich plasma into immunodeficient patients and local transfer of both serum and purified antibodies into nonimmune individuals. These experiments indicated that it was possible to transfer either locally with serum or systemically with plasma, the ability to elicit delayed erythematous lesions. The responses elicited following passive transfer were erythematous but did not have the eczematous features that were seen on patients with atopic dermatitis. Following either systemic or local transfer the lesions elicited were infiltrated with eosinophils. In contrast, basophil infiltration occurred with local transfer of immune serum but not with

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Abbreviations:

BA: binding activity
CBH: cutaneous basophil hypersensitivity
DH: delayed hypersensitivity
IU: international units
LDCF: lymphocyte-derived chemotactic factor
PK: Prausnitz-Küstner

systemic plasma or local purified IgE antibody transfer. The results suggest that the inhalant allergen-induced patch response in allergic individuals may involve a serum factor as well as IgE antibody and the cells normally involved in delayed responses.

MATERIALS AND METHODS

Patients and Plasma

Seventeen patients with diagnosed atopic dermatitis (age range, 18–45 years) who had positive immediate skin tests with *Dermatophagoides pteronyssinus* extract were studied when in remission using a modified patch test technique. All had suffered from severe or moderately severe skin inflammation which was extensive and involved limbs, trunk, and/or face. Twelve of these patients also suffered from bronchial asthma. Five were chosen as plasma and/or serum donors because they were group and cross-match compatible with the plasma recipients. Plasma obtained using a sterile plasmapheresis procedure (Haemonetics Model 30 blood processor), was tested for hepatitis B antigen and stored at -35°C . Five patients with late-onset primary hypogammaglobulinemia consented to be plasma recipients. These patients had normal T-cell numbers and markers [19], as well as normal eosinophil and basophil counts. In addition, 4 who had been tested showed normal T-cell helper function for immunoglobulin production in vitro using pokeweed mitogen and co-culture with normal B cells [19]. The patients with hypogammaglobulinemia had all received plasma infusion in the past as part of their management, and the present infusions were arranged to fit into their treatment schedule. Fifteen nonatopic subjects with negative immediate skin tests and normal total IgE levels, were also studied. Ten of these individuals consented to local transfer studies involving the intradermal injection of atopic serum or purified antibody. All procedures involving human subjects have been passed by the Northwick Park Hospital ethical committee and the subjects were fully informed about the risks involved.

Allergens and Skin Testing Techniques

Antigen P_1 was purified from an extract of *D. pteronyssinus* culture by concentration over an Amicon PM₁₀ filter, fractionation on Sephadex G-100 and pevicon block electrophoresis [20]. Ragweed extract was obtained from Center Laboratories. 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 on the contralateral side of the body. Allergen diluted to $10 \mu\text{g}/\text{ml}$ in normal saline (0.5 ml) was applied on a pad of sterile gauze, occluded with plastic, and retained in position using hypoallergenic tape. After 48 h, the site was inspected, graded, and biopsied under local anesthetic using a 4-mm disposable punch biopsy needle. Care was taken to infiltrate anesthetic around, but not into, the biopsy site. Specimens were fixed in Karnovsky's solution for 24 h, washed in 4 changes of 0.1 M sodium cacodylate buffer, pH 7.4, at 4°C , and dehydrated in varying concentrations of alcohol. Subsequently these biopsy specimens were embedded in hydroxyethyl methacrylate and 1- to $2\text{-}\mu\text{m}$ sections were cut and stained with Giemsa or Azure A [21]. All sections were examined at $630\times$ (Zeiss Planapo lens), and infiltrating cells were identified and counted in the dermis down to the panniculus adiposus. The occasional cells that had migrated into or through the epidermis were not included. Section lengths were confirmed prior to counting and, where necessary, deeper sections were cut to ensure that the section represented the total width of the 4 mm-diameter biopsy specimen.

Following plasma infusion, patches were applied after 2 h and examined after 48 h. After local passive transfer, patches were applied at 24 h (i.e., at the normal time for Prausnitz-Küstner [PK] testing). Intradermal skin tests were carried out using 0.02 ml of antigen P_1 at concentrations of 10^{-6} $\mu\text{g}/\text{ml}$ through $10 \mu\text{g}/\text{ml}$ [22]. Reactions were measured at 15 min and a 6×6 mm wheal was regarded as a 2+ end point. PK testing was carried out by testing the sites with 0.02 ml of 0.1 $\mu\text{g}/\text{ml}$ antigen P_1 24 h later.

Purified Antibodies

Antibodies to antigen P_1 were purified using affinity chromatography as described previously [20]. Antigen P_1 , $10 \mu\text{g}$, was linked to 15 ml of cyanogen bromide-activated Sepharose 4B. This immunosorbent was washed and packed in a $12 \text{ mm} \times 20 \text{ cm}$ column. Two hundred

milliliters of plasma containing high levels of IgE and IgG antibodies to antigen P_1 was passed over the column for 2 h. The column was then washed and eluted with 0.1 M glycine HCl, pH 2.7. The eluted protein was rapidly neutralized with 2.0 M Tris, pH 9.0, and concentrated by negative pressure dialysis.

Antibodies to Antigen P_1 , Total Serum IgE, and Histamine Release

Antibodies to antigen P_1 were measured using class-specific double antibody antigen binding assay [23,24]. The results were obtained from a control curve and expressed in arbitrary units of binding activity (1 unit BA ~ 1 ng). Total serum IgE was measured using double antibody inhibition radioimmunoassay and results are given in international units (IU) [25]. Histamine release was measured by fluorometric assay after 20-min incubation of leukocytes with serial dilutions of antigen P_1 [26].

RESULTS

Eczematous Patch Test Responses to Antigen P_1 in Patients with Atopic Dermatitis

In a series of 17 dust mite allergic patients with atopic dermatitis, patch tests with the purified dust mite allergen antigen P_1 produced a delayed (48 h) eczematous response (++ or +++) in 15 (Table I). These patch tests required $5 \mu\text{g}$ allergen applied for 48 h to mildly abraded skin, whereas immediate wheal-and-flare responses could be produced in these same patients with intradermal injections of nanogram or picogram quantities of allergen (Table I). No delayed phase responses to immediate skin testing were recorded. When the patients were patch tested with saline, only 1 showed an eczematous response (++). Similarly, antigen P_1 on the skin of nonallergic individuals did not produce a response (0/15) (Table I). On biopsy, the eczematous patch tests were shown to be infiltrated with basophils and eosinophils as well as mononuclear cells and neutrophils (Table II). Basophils were identified by their multilobed nuclei, and variable-sized metachromatic granules. Usually there was evidence of degranulation [4]. In contrast, mast cells had a single nucleus and regular, more peripherally distributed granules. These cells did not increase in number in 48-h patch test responses. The mononuclear cells present included monocytes, lymphocytes, and endothelial cells. By contrast, biopsies from 17 saline patches and 3 ragweed patches on these same patients showed very few basophils or eosinophils, and markedly fewer neutrophils and mononuclear cells (Table II).

TABLE I. Macroscopic skin tests and serum antibody levels in atopic dermatitis patients

		Atopic dermatitis		Nonatopic
		Antigen P_1 (n = 17)	Saline (n = 17)	Antigen P_1 (n = 15)
Patch test	+++	5	0	0
responses graded	++	10	1	0
macroscopically ^a	+	2	5	1
	$\pm$ or 0	0	11	14
Intradermal	10^{-4} to 10^{-6}	17	—	0
skin tests	10^{-3} to 10^{-1}	0	—	1
$\mu\text{g}/\text{ml}$ ^b	$>10^1$	0	—	14
Total IgE		5299		39
IU/ml		(3249–8642)		(24–63)
Antibodies to	IgE	189		3
antigen P_1		(98–364)		(3–4)
(binding	IgG	521		4
activity) ^c		(131–2085)		(3–4)

^a 0 = no reaction; $\pm$ = erythema covering $< 10\%$ of the patch area; + = patchy erythema with occasional papules; ++ = confluent papular erythema with or without a mild exudate; +++ = as ++ but with exudation edema and extension beyond the patch area.

^b Using 0.02 ml of serial 10-fold dilutions of antigen P_1 . A wheal $> 6 \times 6$ mm was regarded as the end point. Skin tests negative at $10 \mu\text{g}/\text{ml}$ are recorded as $> 10^1$.

^c The results for IgE and IgG antibodies to antigen P_1 were obtained from a control curve and expressed in arbitrary units of binding activity (1 unit BA ~ 1 ng). Results are expressed as geometric mean values. Figures in parentheses represent 95% confidence intervals around the geometric mean.

The 3 patients tested with ragweed antigen were previously shown not to have immediate hypersensitivity to this antigen.

Systemic Passive Transfer into Patients with Hypogammaglobulinemia

Plasma obtained from 5 of the atopic dermatitis patients was transfused into 5 group- and cross match-compatible patients with primary hypogammaglobulinemia. Each patient received 3 units of plasma intravenously over a 4-h period; no transfusion reactions were observed. The recipients had very low levels of serum IgE prior to infusion and reached levels of 553–3800 IU/ml (Table III). Specific antibodies rose in parallel with serum IgE (Table III). Over the 48 h after infusion, total and specific IgE in the serum fell by > 50%. In each recipient immediate skin reactivity became demonstrable as early as 2 h post infusion, maximal at 24 h, and remained constant over the next 7 days (Table IV and data not shown). In addition, 4 out of 5 recipients gave positive histamine release from peripheral blood leukocytes with antigen P₁ at 1 and 48 h after infusion (Fig 1). No significant differences were noted between these two time points. Patch testing post infusion showed erythematous responses in each of the recipients (Table IV). However, 2 of the 5 recipients had shown positive responses prior to infusion. These patch responses post infusion were qualitatively different from those seen in the plasma donors as they showed few papules and had less induration and exudation.

Microscopically, neither eosinophils nor basophils were present in the preinfusion patch test biopsies. Postinfusion, the patch test responses in all 5 patients showed pronounced eosin-

ophil infiltration but basophil infiltration was not present in any of these biopsies (Table V).

Passive Local Transfer into the Skin of Nonallergic Individuals

Serum was obtained from 3 subjects with atopic dermatitis who were known to recruit basophils to an antigen P₁ patch test on their own skin. These sera, which gave positive PK tests at a dilution >1:2000 were used at 1:2 dilution and 0.05 ml was injected intradermally into 7 nonatopic control subjects. Antigen P₁ patch tests were applied to these sites after 24 h and examined 48 h later. The patch tests gave negative or weakly positive macroscopic erythematous reactions; however, on biopsy, a significant infiltrate of both eosinophils and basophils was present (Table V). The number of basophils present was comparable to that seen in patch tests on patients with atopic dermatitis (Table I). Three types of controls were used for local passive transfer. Use of saline injections followed by antigen P₁ patch tests did not give rise to basophil or eosinophil recruitment. If the serum was heated at 56°C for 2 h prior to injection, the subsequent response to antigen P₁ was abolished. Furthermore, in two patches after serum transfer, ragweed extract was used instead of antigen P₁, and this did not produce a significant basophil or eosinophil infiltrate.

The results of patch testing following the heating of serum might be thought to imply that the activity in serum was associated with IgE antibody. However, we had previously seen that systemic transfer, which gave rise to marked immediate hypersensitivity, did not allow basophil recruitment. In order to investigate the role of IgE antibody further, we used specifically purified IgE and IgG antibodies to antigen P₁ for local

TABLE II. Differential cell count of infiltrating cells in biopsies from patch test sites^a

	Atopic dermatitis subjects			Non-atopic subjects
	Antigen P ₁ (n = 17)	Ragweed (n = 3)	Saline (n = 17)	Antigen P ₁ (n = 15)
Basophil	26 (4–214)	1 (1–8)	1 ^b (0–29)	0 ^b (0–23)
Eosinophil	104 (24–1786)	17 (5–24)	7 ^c (0–89)	1 ^b (0–5)
Mast cell	60 (19–174)	74 (56–74)	56 ^d (24–144)	68 ^d (23–97)
Mononuclear cell	727 (368–1254)	304 (296–415)	339 ^e (138–658)	231 ^c (115–909)
Neutrophils	37 (12–283)	14 (8–23)	16 ^d (3–205)	8 ^c (0–62)

^a Histologic sections examined were of similar size (4-mm width). Where necessary, further sections were cut to ensure comparability. All infiltrating cells in the dermis were counted. Results are expressed as median values and the range. Within-subject comparisons between the number of each cell type infiltrating the lesions induced with antigen P₁ and saline were made by means of the sign test; between-subject comparisons of antigen P₁-induced lesions were made by means of the Mann-Whitney U-test [27].

^b $p < 0.0001$.

^c $p < 0.0005$.

^d Not significant.

^e $p < 0.0025$.

TABLE III. Antibody levels (pre and post plasma infusion)^a

Plasma recipients	Total IgE antibody (IU/ml)				Specific antibody (BA/ml)					
	Donor plasma	Pre infusion	Post infusion (1h)	Post infusion (48h)	IgE anti-P ₁			IgG anti-P ₁		
					Donor plasma	Post infusion (1 h)	Post infusion (48 h)	Donor Plasma	Post infusion (1 h)	Post infusion (48 h)
1	11,600 ^b	10	3,800	1,650	564	51	20	141	49	38
2	6,400	42	553	233	103	41	18	165	36	22
3	9,900 ^b	9	1,835	808	281	28	8	216	18	9
4	8,900	6	837	425	196	54	24	225	29	21
5	7,300 ^b	4	2,101	1,222	768	200	133	2,962	485	334

^a Total IgE levels and specific P₁ antibodies of both IgG and IgE isotypes were measured before and at 1 and 48 h after infusion.

^b Sera from these 3 atopic dermatitis patients were also used in local transfer experiments.

TABLE IV. Skin test responses after passive transfer

	Patch tests with antigen P ₁ (10 µg/ml for 48 h)		Intradermal skin tests ^a (µg/ml at end point)
	Pre	Post	Post
Systemic plasma (5)	± ++ Neg Neg +	++ +++ + + +	10 ⁻² 10 ⁻³ 10 ⁻⁴ 10 ⁻⁵ 10 ⁻⁴
Local serum ^b (7)	No heat		Heat
	Neg	+	–
	Neg	±	–
	Neg	±	ND
	Neg	±	ND
	Neg	±	±
	Neg	+	ND
	Neg	±	ND

^a Performed using 0.02 ml of serial 10-fold dilutions of antigen P₁. A wheal > 6 × 6 mm was regarded as the end point. All subjects were skin test-negative (both prick and intradermal at 10 µg/ml) prior to transfer. ND represents not done.

^b These sera gave positive PK tests at >1:2000 dilution; 0.05 ml of a 1:2 dilution was used.

transfer (IgE ml). Five non P₁-specific and h immediate µg/ml antigen mast cell sens that seen in t at the remain erythematous When these lo significant eos infiltrate (Tal

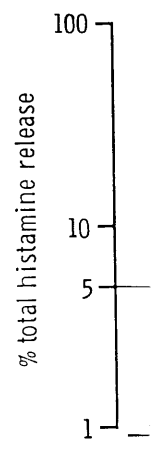


FIG 1. Leuk

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< 5% histamin

Transfer

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Preplasma

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^b $p < 0.016$.

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^e Serum was

transfer (IgE ab: 10,479 BA units/ml; IgG ab: 8,254 BA units/ml). Five nonatopic control subjects received 0.05 ml of antigen P₁-specific antibody intradermally at 4 different sites. After 24 h immediate wheal responses (> 6 × 6 mm) using 10⁻³ to 10⁻⁴ µg/ml antigen P₁/ml were elicited at 3 of these sites. Thus, mast cell sensitization with IgE antibody was comparable to that seen in the patients. Patch testing with antigen P₁ (5 µg) at the remaining site, 24 h after injection, resulted in positive erythematous macroscopic responses 48 h later (Table IV). When these lesions were biopsied, they were found to have a significant eosinophil infiltrate; however, there was no basophil infiltrate (Table V). In Table V the mononuclear cell counts

are also shown. These reveal a higher number of mononuclear cells in patches after systemic and local transfer. However, in general, there was greater background variability with both mononuclear cells and neutrophils than with eosinophils and basophils.

DISCUSSION

Using either systemic plasma infusion, local serum, or local purified antibody, it has proved possible to transfer the ability to elicit immediate and "delayed in time" reactions of the skin. Immediate hypersensitivity was demonstrable by intradermal skin testing, and also after systemic transfer, by the demonstration of histamine release from peripheral blood leukocytes. The delayed skin lesions demonstrated on the plasma recipients after 48-h patch tests were erythematous, and in several cases, this erythema was as intense as that seen in patches on the donors. The macroscopic reactions to patch testing following local serum or antibody transfer were generally very weak. Although we elicited delayed erythematous reactions following transfer, these reactions lacked the other macroscopic features of eczema. Thus, it has not proved possible to passively transfer the full lesion that can be demonstrated on patients with atopic dermatitis.

The biopsies from antigen P₁ patches on patients with atopic dermatitis, have the features of CBH, with basophils, eosinophils, and mononuclear cells infiltrating the dermis. Following both systemic and local transfer, the presence of an eosinophil infiltrate in the antigen P₁ patch tests correlated closely with the transfer of immediate hypersensitivity. Thus, the eosinophil infiltrate may be explained simply by the release of mediators from passively sensitized mast cells in the skin. The modest increases in mononuclear cells and neutrophils may also be explained on this basis. In the transfer experiments, basophil recruitment was seen only after local transfer of unheated serum. The failure of systemic transfer or local antibody to recruit basophils suggests strongly that sensitization of mast cells with IgE antibody is not sufficient on its own to recruit basophils. This finding is in keeping with the fact that basophils are not present in skin lesions induced by histamine injection or 48/80-induced mast cell degranulation [28]. Also, mediators released from mast cells and basophils have not been demonstrated to have basophil chemotactic activity. While it is clear that basophils are present in late-phase skin responses, it is

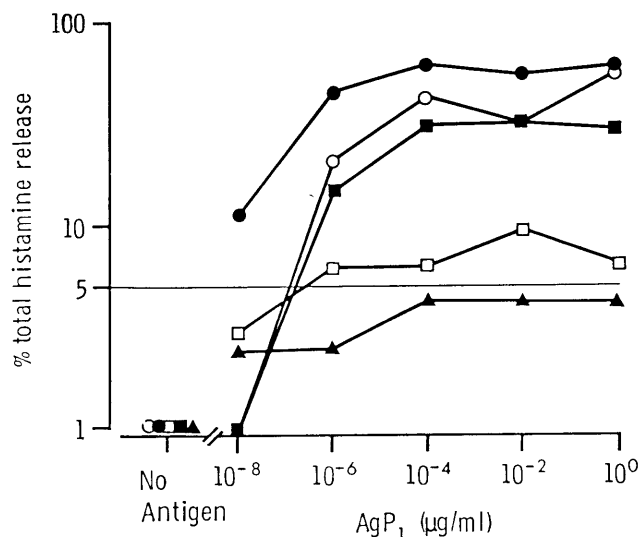


FIG 1. Leukocyte histamine release 48 h after plasma infusion.

% Total histamine release =

$$\frac{(\text{Histamine released by antigen P}_1 \text{ stimulation} - \text{Background release of histamine}) \times 100}{\text{Histamine released by incubation at } 100^\circ\text{C}}$$

Each symbol represents 1 patient. Preinfusion of all 5 subjects showed < 5% histamine release.

TABLE V. Cells infiltrating patch test sites following passive transfer

Transfer	No.	Antigen used in patch test	Differential cell count on biopsy section (mag × 630)*				
			Basophil	Eosinophil	Mast cell	Mononuclear cell	Neutrophil
<i>Systemic</i>							
Preplasma	5	Antigen P ₁	0 (0-1)	1 (0-5)	61 (20-86)	296 (164-665)	16 (2-254)
Post plasma	5	Antigen P ₁	1 (0-4)	145 (3-535)	47 (32-93)	339 (162-797)	47 (25-409)
<i>Local</i>							
Saline	8	Antigen P ₁	0 (0-4)	1 (0-2)	44 (23-92)	206 (129-263)	6 (0-31)
Serum	7	Antigen P ₁	69 ^b (12-161)	320 ^b (20-560)	82 ^c (46-87)	415 ^c (165-741)	59 ^c (3-200)
Heated serum ^e	2	Ragweed ^d	(2-4)	(4-30)	(80-113)	(264-541)	(11-27)
	3	Antigen P ₁	6 (0-6)	0 (0-10)	63 (29-108)	327 (106-347)	2 (2-56)
Purified antibody	5	Antigen P ₁	0 (0-7)	99 (45-188)	34 (21-41)	347 (237-606)	19 (13-50)

Statistical analysis between the number of each cell type infiltrating the lesions induced with antigen P₁ and saline following local serum transfer was made using the sign test. No significant testing was done on groups comprising 5 or fewer subjects.

* Histologic sections were of similar size (4-mm width) and all infiltrating cells in the dermis were counted. Results are expressed as median values and the range.

^b $p < 0.016$.

^c Not significant.

^d Ragweed extract was obtained from Greer Laboratories Inc. Lenoir, North Carolina.

^e Serum was heated at 56°C for 3 h.

not clearly shown that basophils are recruited to the delayed cutaneous reactions that can be elicited following transfer of IgE antibody [8]. In the present experiments, the ability of immune serum to mediate basophil recruitment was abolished by heating. However, it is possible that basophil recruitment requires IgE antibody-induced mediator release and another factor. Our findings contrast with results in guinea pigs where recruitment of basophils to a skin site has been seen after systemic transfer but not after local transfer [17]. We estimate that the quantity of plasma that we used for systemic transfer was 6-fold greater on a weight:volume ratio than the minimum quantity of immune serum necessary for systemic transfer of CBH to a guinea pig. We consider it unlikely that the differences between systemic and local transfers were due to the use of plasma in one case and serum in the other. Also the difference in the time of application of patch tests following the respective procedures was unlikely to explain the absence of basophil recruitment in the case of systemic transfer.

The mechanism for basophil recruitment and accumulation in hypersensitivity lesions is unknown. In experimental animals, basophil recruitment is T cell-dependent. Even when serum is used to transfer CBH, T cells in the recipient may play an active role [6]. In humans, the only established basophil chemotactic factors are lymphocyte-derived chemotactic factor (LDCF) and the activated complement component C5a [29,30]. Recently, we have found that peripheral blood basophils from patients with atopic dermatitis will respond to both these agents in vitro [31]. It is possible that immune serum contains a factor that can mediate basophil recruitment. This factor could act cytophilically to release a basophil chemotactic factor from a secondary cell or might be involved in the recruitment of T cells which were in turn induced to release LDCF. If there is a serum factor responsible for basophil recruitment, then we have to assume that this factor does not accumulate in the skin following systemic transfer of plasma. Whether it is possible that this lack of accumulation is due to the putative factor having a large molecular size and thus not entering the tissues has not been determined. It is also possible that IgG antibody with or without complement activation contributed to the cell infiltrates observed. Certainly, IgG antibody to antigen P₁ was present in all the transfer situations. It seems unlikely that IgG antibody contributed to basophil accumulation since this activity was heat-labile and was not mediated by the purified antibody which contains high levels of IgG antibody. However, in guinea pigs low-affinity IgG antibody can transfer basophil accumulation, and our specific antibody preparations may not fully reflect the affinities of the IgG antibody in the whole serum. Also, it has been reported that a large proportion of IgG antibody to allergens is of the IgG₄ subclass and would not be expected to fix complement [32]. At the present time it remains unclear whether an IgG subclass antibody, a serum factor, possibly antigen-specific, or other serum component mediates the basophil recruitment that occurs at the patch test sites.

The presence of basophils in allergen-induced patch test reactions on patients with atopic dermatitis suggests that these lesions represent a form of DH. However, our results with local injections of serum suggest that the sera of these patients contain an antigen-specific activity that can regulate basophil accumulation and does not appear to be IgE antibody. The role of the basophils in patch test responses remains unclear. It is possible that mediators released locally by basophils have the capacity to augment the inflammatory response as has been demonstrated in guinea pigs [33,34]. In addition, mediators, such as histamine, are known to have immunoregulatory functions which may be involved in the patch test reactions [35]. The lesions produced by patch testing after passive transfer were not equivalent to the lesions seen on patients with atopic dermatitis. Fibrin deposition, seen in delayed reactions [36], was present under electron microscopy in some of the patch

test responses elicited in atopic dermatitis patients and was particularly marked in those showing strong reactions with high numbers of basophils present. No consistent pattern of fibrin deposition was noted in the patch tests following passive transfer. We believe that sensitized T cells may play a role in the patch tests on patients with atopic dermatitis. It is well established that there is a T-cell infiltrate in atopic eczema, and the patch tests on patients had a significant mononuclear infiltrate. Furthermore, we have recently proved that T cells from these patients will proliferate in vitro in response to antigen P₁ [37].

The cell infiltrate in the patch reaction is not the same as that reported in chronic lichenified eczema [38]. Recently, we have observed that repeated application of antigen for up to 10 days on patients with atopic dermatitis will produce eczematous lesions which more closely represent the histologic picture of chronic eczema. In these lesions, eosinophil infiltration is less and mast cell hyperplasia replaces basophil infiltration. Mast cell hyperplasia replacing early basophil recruitment has been demonstrated in other forms of basophil hypersensitivity in humans [39]. Whether the increased mast cell number that occurs in the skin represents a heterogeneous population of mast cells remains unclear. In experimental animals, hyperplasia of mucosal mast cells and of cloned mast cells has been shown to be T cell-mediated [40,41]. It appears that basophil arrival at delayed reactions is under complex control and the role of mast cells and basophils in these lesions may be complementary. Taken together our results suggest that the development of an eczematous reaction on the skin may well involve eosinophils, basophils, IgE antibody, and other serum factors as well as a role for specifically sensitized T cells. The present experiments may well shed further light on the mechanisms of inflammation in naturally occurring atopic dermatitis.

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