

A STUDY OF CELLULAR RESPONSES IN IMMUNE REACTIONS UTILIZING THE SKIN WINDOW TECHNIQUE

I. Immediate Hypersensitivity Reactions

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IT has recently been demonstrated in this laboratory that the application of the allergen to the skin of individuals exhibiting hypersensitivity of the immediate type results in the production of an intense eosinophilia, as observed by the skin window technique.¹ In addition, eosinophilia can be successfully transferred to normal nonallergic individuals by transfer of sera containing reaginic activity, as demonstrated by the eosinophilia in the skin window performed at the site of the serum transfer.¹

In the present work, the sequence of cellular events which characterizes allergic inflammation of immediate hypersensitivity has been investigated in greater detail. In view of the eosinopenia produced by administration of glucocorticoids² and the amelioratory effect of such steroids in a large number of allergic conditions,³ it seemed of interest to study the effect of oral as well as topical steroid on the skin window eosinophilia. The effect of antihistamines on skin window eosinophilia was also investigated. Finally, the cellular response to some of the suspected chemical mediators of allergic reactions was studied in normal and allergic subjects.

MATERIALS AND METHODS

Skin Window Technique.—Skin windows were made in 20 allergic patients, all of whom gave a clinical history of allergic rhinitis with strongly positive skin tests to ragweed, grass, tree, or fish allergens and six of whom had also experienced asthmatic attacks. The method employed was that of Rebuck and Crowley.⁴ Following staining and mounting of the cover slips, differential cell counts were made on a total of 500 cells, when available.

Effect of Drugs.—*Antihistamines:*† Phenergan, 50 mg. every 6 hours, was given orally to 10 allergic volunteers for 24 hours prior to the skin window test

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and throughout the period of the test (24 hours). The drug was given in the same dose to 4 normal subjects as controls.

Topical Steroid: 6-Methylprednisolone* ointment was applied to the skin of the forearm of 5 allergic patients in liberal amounts 4 to 6 times per day for 2 days prior to the skin window test. The cellular response to the allergen was compared to the response in the opposite untreated arm.

Oral Steroid: 6-Methylprednisolone, 8 mg. every 6 hours, was given for 2½ days to 5 allergic volunteers. Skin window tests were performed for the 24 hour period beginning after the second dose of steroid, and a second set was performed at new sites on the forearm following the sixth dose of steroid. Blood eosinophil counts were done prior to the initial dose and after the second and sixth doses corresponding to the time at which the first and second skin window tests were started.

Chemical Mediators: Several of the suspected chemical mediators of allergic reactions were dissolved in saline and utilized in skin window tests. Histamine HCl, 1 drop of a solution containing 100 µg per cubic centimeter, bradykinin, 1 drop of a solution of 200 µg per cubic centimeter, and serotonin (hydroxy-tryptamine creatinine phosphate), 1 drop of a solution of 1,000 µg per cubic centimeter, were applied separately to the prepared sites on 10 allergic subjects and 6 normal control volunteers. The cellular response at 1, 2, 4, 6, 12, and 24 hours was then examined and compared with the response of saline or toxoid control sites.

Allergic Responses in a Cantharides Blister.—Cantharides, 0.7 per cent contained in a solution of acetone and collodion, was applied as a vesicant to the skin of the forearm of 6 allergic and 4 normal individuals. Eighteen hours later, 0.1 c.c. of the ragweed, grass, or tree extract was injected directly into the cavity of the blister which had formed. Injections were performed with a No. 26 needle, the needle tip being placed beside the blister and the blister cavity entered from below. The blisters were punctured at various time intervals following injection of the allergenic material. The blister fluid obtained was centrifuged at 1,500 r.p.m. and the supernatant removed. The small button of cells thus obtained was resuspended in a small amount of blister fluid and streaked on slides. Control blisters on the opposite arm which had been injected with 0.1 c.c. of diphtheria or tetanus toxoid were similarly treated. Following evacuation of the blister, the roof was removed and a glass coverslip applied to the denuded site which formed its base. In this way a tissue cell sample could be obtained when the blister was evacuated as well as a sample of the cells in the blister fluid.

RESULTS

The Cellular Events.—Table I summarizes the cellular events in a skin window stimulated by the application of ragweed allergen compared to those in a control site to which tetanus toxoid had been applied in 1 allergic subject. The control site was characterized initially by the accumulation of polymorphonuclear leukocytes, with mononuclear cells in the minority. Subsequently, however,

*Provided by the Upjohn Company, Toronto, Canada.

the infiltration with leukocytes diminished while the mononuclear cells accumulated strikingly so that at 24 hours the infiltrate consisted mainly of mononuclear cells (Fig. 1). Eosinophils were occasionally noted in the early hours of the skin window response. A rare basophil was noted, usually at the 24 hour sampling. In contrast, the allergic site was characterized by the accumulation of eosinophils, which even in the first 6 hours were more numerous in the test

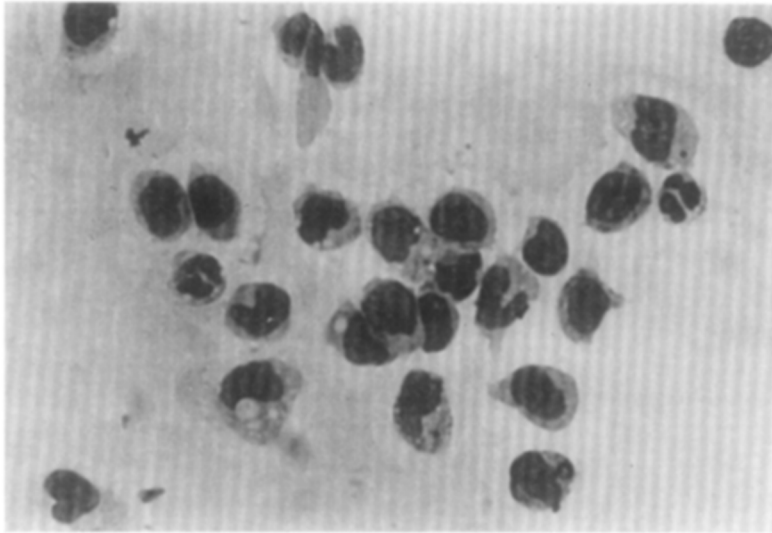


Fig. 1.—Cellular infiltrate at 24 hours of the control site. Note the predominance of mononuclear cells. ($\times 630$.)

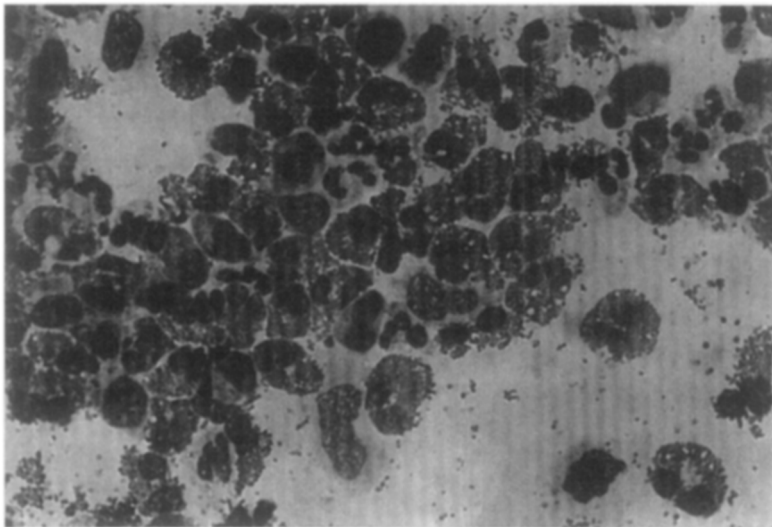


Fig. 2.—Cellular infiltrate at 24 hours of the allergic skin window site. The eosinophilia is striking. Many cells have disrupted, spewing forth granular material. A smaller number of polynuclear and mononuclear cells are also visible. ($\times 500$.)

site than in the control. Subsequently, however, these cells accumulated in very large numbers, often being the predominant cell type at the 24 hour period (Fig. 2). The polymorphonuclear leukocyte response did diminish but not to the same extent as in the control site. The mononuclear cell response was much less marked, so that at 24 hours it was present as a minority of the cells.

In the 20 allergic subjects, the cellular responses of the test site to which a drop of the appropriate allergen had been applied exhibited wide variations in numbers of the various cell types. Eosinophil counts varied from 3 to 60 per cent of cells at 24 hours. The mononuclear cell infiltrate was not invariably diminished at 24 hours, since these cells often comprised 70 to 80 per cent of the infiltrate, as in the control site (Table I). The expected (high) mononuclear cell response was noted more frequently when the eosinophilia was less than 10 per cent.

In view of the predominance of the eosinophil at 24 hours in the skin window, subsequent studies of the allergic response centered upon the alterations in numbers of eosinophils at this time. The 24 hour skin window response was then studied in a group of 5 patients exhibiting symptoms in ragweed season only but in whom the skin reaction to grass as well as ragweed could be classified as a 4 plus reaction. The results given in Table II indicate that the eosinophilia in all 5 patients could be correlated with the clinically allergenic material in that the eosinophils in the skin window site stimulated by ragweed were present in much greater numbers than in the grass-stimulated site.

Effect of Drugs on the 24 Hour Skin Window Response.—Table III summarizes the findings in a group of 5 patients in whom skin windows were performed following the ingestion or application of antiallergic drugs. Antihistamines (Phenergan) given systemically appeared to increase the eosinophilia noted at 24 hours in the skin window in the small group of allergic subjects, but it did not alter the cellular progression in 4 normal individuals. 6-Methylprednisolone applied topically did not produce a consistent alteration in tissue eosinophilia, since in some instances eosinophil levels increased while in others they decreased. Skin window eosinophilia was completely or almost completely abolished by the ingestion of oral steroid, and, as expected, the blood eosinophil counts were markedly reduced when compared with the normal eosinophil count prior to the ingestion of the drug.

Allergic Reactions in a Cantharides Blister.—The cellular events in a blister were similar to those noted in the skin window, with a few differences. The numbers of mononuclear cells in the test site as well as the control were generally fewer at 24 hours than in the skin window (Table IV). In addition there were a number of bizarre cells that could not be identified. Following injection of the allergen, the eosinophilia was very striking. Eosinophils were present in large numbers, totaling 50 per cent of cells in many instances, in the 24 hour fluid sample from the test site (Table IV). In addition, application of a glass coverslip to the denuded allergic blister site, which presumably would be a reflection of the cells accumulating in the skin at the time of application of the coverslip, frequently revealed extremely large numbers of eosinophils. In 2 patients the

TABLE I. DIFFERENTIAL CELL COUNTS ON 500 CELLS IN AN ALLERGIC SUBJECT

TIME	CONTROL SITE			RAGWEED TEST SITE		
	POLYS	MONOS	EOS	POLYS	MONOS	EOS
2 hours		Very few cells		98	0	2
4 hours	76	24	1	94	3	3
6 hours	64	35	1	89	8	3
8 hours	63	31	1	87	9	4
10 hours	56	44	0	82	6	12
12 hours	48	52	0	78	6	16
24 hours	16	84	0	50	6	44

TABLE II. DIFFERENTIAL CELL COUNTS OF 24 HOUR RESPONSES IN PATIENTS EXHIBITING SYMPTOMS IN RAGWEED SEASON

PATIENT	CONTROL SITE			GRASS-STIMULATED SITE			RAGWEED-STIMULATED SITE		
	POLYS	MONOS	EOS	POLYS	MONOS	EOS	POLYS	MONOS	EOS
D. T.	49	51	0	32	53	15	30	34	36
T. M.	14	86	0	24	57	19	44	8	48
R. O.	93	6	1	55	39	6	38	41	21
G. B.	56.6	40.8	2.6	96.8	2.8	0.4	73.2	14.3	12.5
H. C.	41	59	0	42	54.8	3.2	61	22.2	16.8

TABLE III. SKIN WINDOW RESPONSE AT 24 HOURS TO RAGWEED ALLERGEN, PER CENT EOSINOPHILS PER 500 CELLS

TREATMENT	PATIENT				
	1	2	3	4	5
Ragweed control	9 (426)	14 (373)	16 (513)	54 (418)	52 (193)
Antihistamine	36	18	40	68	60
Topical steroid	21	9	6	46	24
Oral steroid I	10 (68)	0 (104)	4 (84)	0 (87)	2 (87)
II	2 (30)	0 (68)	0 (44)	0 (50)	0 (43)

Figures in parentheses = blood eosinophil counts per cubic centimeter.

eosinophil counts in such skin windows numbered 90 per cent of the cells on the coverslip at 24 hours. The percentage of eosinophils in the control blister fluid at 24 hours (Table IV) was greater than the percentage observed in the corresponding skin window control site (Tables I and II). However, skin windows performed on the denuded blister sites at 24 hours revealed fewer numbers of eosinophils than in the control fluid, suggesting that the high percentage in the fluid sample was the result of accumulation of these cells throughout the 24 hours rather than of a representative sample at the 24 hour stage. The injection of steroid into the blister cavity 15 minutes prior to the allergen did not produce a consistent alteration in the cellular infiltrate or in the skin window on the denuded blister site. By contrast, systemic steroid completely abolished blister fluid and tissue eosinophilia while in agreement with the results of the skin window studies.

Effect of Known Chemical Mediators.—An alteration of the normal cellular

TABLE IV. DIFFERENTIAL CELL COUNTS IN 24 HOUR BLISTER FLUID SAMPLES

PATIENT	CONTROL FLUID			TEST FLUID		
	POLYS	EOS	MONOS	POLYS	EOS	MONOS
1	56	9 (5)	35	72	18 (12)	10
2	45	10 (1)	45	25	32 (47)	43
3	78	3 (6)	19	56	25 (32)	19
4	60	16 (6)	24	38	51 (46)	11
5	85	7 (6)	8	68	22 (52)	10
6	24	32 (8)	44	47	50 (90)	3

Figures in parentheses denote percentage of eosinophils observed in skin windows performed at denuded blister sites.

progression was noted in 9 of the 14 allergic subjects following application of approximately 5 μ g of histamine—HCl to the skin abrasion. The cellular response began earlier with appreciable numbers of polys noted at 1 hour, in contrast to the relative absence of cells in the control site. Some eosinophils were noted in the test site at 1 hour. The peak, however, occurred at 2 to 4 hours, and cell counts at that time demonstrated a range of 8 to 45 per cent eosinophils in the 9 subjects, as compared to only 3 per cent in the control site in 8 of 9 individuals. In the single instance in which the test site exhibited an eosinophilia of 45 per cent, the control site demonstrated 9 per cent eosinophils at 4 hours. The eosinophilic cellular infiltrate then diminished markedly and was followed by the expected cellular response. Bradykinin and serotonin did not alter the normal cellular progression, although the latter in 2 allergic subjects was associated with the development of an eosinophilia of 5 and 9 per cent at 24 hours.

The histamine-induced eosinophilia tended to be greater in subjects exhibiting allergic or suspected allergic skin manifestations compared to subjects with allergic rhinitis alone. This agent, as well as the others tested, did not induce eosinophilia in any of the 10 normal, nonallergic subjects.

DISCUSSION

The cellular events in acute inflammation produced by a simple abrasion of the skin were previously investigated by Rebuck and Crowley.⁴ The two major events in the first 24 hours were an initial infiltration of polymorphonuclear leucocytes, followed by a late infiltration of mononuclear cells. In the present work, application of the allergen to the abrasion or its injection into a prepared blister cavity in the allergic subject initiated an inflammatory response upon which a local allergic reaction to the allergen was superimposed. The second reaction was characterized by a variable, often intense, infiltration with eosinophils. The accumulation of eosinophils was greatest at 24 hours, long after the intradermal response to the allergen had ended. Moreover, the eosinophilia was greatest when elicited by the allergen related to the patient's symptoms. This was shown by the greater eosinophilia to ragweed than to grass extract in 5 subjects with symptoms only in late summer but with similar 4 plus intradermal skin tests to both materials.

The antiallergic drugs differed in their influence upon skin window eosinophilia. Phenergan appeared to increase the eosinophilic infiltrate. However, since

daily tests in a small group of allergic subjects demonstrated variation in numbers of eosinophils by as much as 50 per cent, further studies utilizing a larger group of subjects are required before it may be concluded that phenergan actually increases eosinophilia. In any event, the results of the present work agree with the work of Samter,⁵ who demonstrated that these agents were not effective in reducing the eosinophilia induced by antigen or histamine in sensitized guinea pigs even at a dosage which protected the animals from fatal anaphylaxis. Few and inconsistent effects were observed with topical steroid. In contrast, oral steroid markedly or totally abolished tissue eosinophilia, presumably by reducing the blood eosinophil level, thereby diminishing the supply of eosinophils available to infiltrate an allergic tissue. The possibility of a local effect of the steroid, in which tissue eosinophilia is reduced as a consequence of the local reaction to the agent, has probably not been ruled out in the present work.

The factors mediating eosinophilotactic activity in allergic reactions have been the subject of many investigations. Kline and co-workers,⁶ utilizing a biopsy technique in human subjects, demonstrated the rapid influx of eosinophils into the site of the wheal stimulated by the intradermal injection of allergen. The infiltration could be duplicated by intradermal injection of histamine alone in allergic individuals and not in normal, nonallergic subjects. Archer⁷ more recently demonstrated the accumulation of eosinophils in the intradermal injection site of horses upon injection of histamine. The number of infiltrating eosinophils increased with increasing dosage of histamine. The amount injected was greater than the potential quantity which could be liberated by an allergic reaction, evidence favoring the view that other mediating factors as yet unknown must play a role in inducing tissue eosinophilia.

The behavior of blood eosinophil levels following histamine injection is similar to the picture in tissues. Samter⁵ demonstrated the development of blood eosinophilia in guinea pigs sensitized to horse serum and not in normal nonsensitized animals 20 hours after the intracardiac injection of 75 μ g of histamine, a large nonphysiologic dose. Litt⁸ injected histamine into the peritoneal cavity of normal guinea pigs but failed to reproduce the eosinophilia noted following the injection of antigen into the peritoneal cavity of sensitized guinea pigs. He did not report any findings on injection of histamine into animals sensitized with foreign protein.

Our experiments have utilized a human model in which the allergic tissue was sensitized with antibodies of the skin-sensitizing type. In this instance, application of the allergen produced a slowly progressive infiltration, with a peak accumulation 24 hours after the stimulus and long after the response to the corresponding intradermal injection of allergen had vanished. In contrast, histamine in allergic but not in nonallergic subjects initiated an early eosinophilia (within 4 hours of the stimulus) which diminished or vanished at 24 hours. The discrepancy in the time of peak eosinophilia observed with the allergen and histamine cannot be explained as yet. It may be that the application of the allergen to the skin abrasion induces a more prolonged response in the skin window instead of the short acute response of the intradermal injection. Thus the chemical mediators of eosinophilia probably are released gradually

rather than abruptly. Histamine alone therefore could exert an immediate response because it is rapidly inactivated. In addition, the finding that histamine was effective in inducing eosinophilia only when applied to allergic tissue indicates at least one additional property possessed by allergic tissue but not normal skin. Since eosinophilotactic activity could be transferred by conventional passive transfer of allergic serum and since this activity was abolished by heating the serum to 60° C., it would appear that the factor(s) responsible for inducing eosinophilia must be closely related to, if not identical with, skin-sensitizing antibody. This problem is being investigated.

Histamine-HCl was utilized in our studies in a dosage of approximately 5 μ g contained in 1 drop applied to the skin abrasion. This dose is probably greater than any which could be released by an allergic response.⁸ However, since a glass coverslip applied to the drop results in a thin spread of fluid under the entire glass, it cannot be determined with certainty how much of the 5 μ g is in fact exerting a response at the skin abrasion site. Thus it is possible that this dose represents a pathophysiologic stimulus. If so, it may be concluded that histamine is at least one of the chemical mediators of eosinophilotactic activity in allergic reactions in humans with some types of immediate hypersensitivity. In this connection, attempts to demonstrate eosinophilia following application of the antigen to the skin abrasion in 2 individuals with immediate-type hypersensitivity to tetanus toxoid with wheal-and-flare response were unsuccessful.⁹

These initial studies suggest the potential usefulness of this technique in assessing the allergic properties of several types of clinical disease states.

SUMMARY

Tissue eosinophilia has been demonstrated in the skin sites of allergic individuals following application of the allergen. The eosinophilia was greatest with the allergen which clinically seemed to be responsible for the patient's symptoms. Phenergan appeared to increase skin window eosinophilia, although this conclusion must await further study. Application of a topical steroid failed to produce a consistent alteration in eosinophil counts, while oral steroid therapy abolished eosinophilia. The steroid probably exerted its effects by markedly reducing the blood eosinophil level. The suspected chemical mediators of eosinophilia in a skin abrasion were investigated. These studies demonstrated that histamine was capable of inducing tissue eosinophilia in allergic subjects but not in normals, implicating skin-sensitizing antibody as a further prerequisite for the production of tissue eosinophilia.

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