

Effects of injections of allergen extracts on antigenic release of histamine from leucocytes, skin tests and bronchial challenge in allergic children

MICHAEL J. SCHUMACHER and CHARLES D. MAY

*Pediatric Allergy Division, National Jewish Hospital and Research Center, and
Department of Pediatrics, University of Colorado Medical School, Denver, Colorado*

Summary

Leucocyte sensitivity to antigenic release of histamine was determined in allergic children receiving injections of allergen extracts in order to ascertain the frequency of development of complete loss of sensitivity (unresponsiveness). Leucocyte unresponsiveness did not occur in any of eight allergic children given large doses of allergen extracts by an intensive regimen of injections. In only two of twenty-three children who had received injections of allergen extracts by a customary dosage regimen, were the leucocytes found to be unresponsive to antigenic histamine release. These findings confirm our earlier reports.

The incidence of complete loss of leucocyte sensitivity during injection therapy in our studies is lower than reported by others. This is attributed to our employment of a more nearly optimal *in vitro* system for measurement of antigenic histamine release from leucocytes, thus avoiding the false impression of leucocyte unresponsiveness which can result from use of a less sensitive procedure.

Other findings in the present investigation were: (1) concordant fluctuations in leucocyte sensitivity to two unrelated allergens during injections of one of the allergens, indicating non-specific changes in sensitivity; (2) skin sensitivity and bronchial reactivity were not reduced even with marked decrease in peripheral leucocyte sensitivity; (3) significant decrease in titre of serum complement, as occurs in presence of antigen-antibody complex reactions.

Introduction

Rational use of injections of allergen extracts for the treatment of allergic disorders has been hampered by limitations in knowledge of its effect on basic immunochemical responses. In recent years, expectations have been raised that substantial improvement in the understanding and practical use of allergen injections may come from laboratory measurements coupled with controlled clinical trials of various dosage regimens. Some investigators (Sadan *et al.*, 1969; Levy *et al.*, 1971) derived encouragement from their observation of complete loss of sensitivity of leucocytes to antigenic histamine release

Correspondence: Dr Charles D. May, Pediatric Allergy Division, National Jewish Hospital, 3800 E. Colfax Avenue, Denver, Colorado 80206, U.S.A.

in a small number of subjects during injection therapy. In contrast, complete loss of leucocyte sensitivity during injection therapy has been rare in our experience (May *et al.*, 1972a).

One objective of the present study was to enlarge our experience to determine more precisely the incidence of occurrence of complete loss of leucocyte sensitivity during injection therapy. Two groups of children were studied. In a prospective study, an intensive regimen of injections was used to provide large dosage of allergen extract in a short period. By a retrospective study, in which the status of leucocyte sensitivity could be determined after customary injection therapy had been administered, supplementary information could be gathered relatively quickly on the likelihood of more prolonged treatment achieving complete loss of leucocyte sensitivity.

Other objectives of the present study were: (1) to examine relationships between responses to allergen measured by leucocyte histamine release, skin tests and bronchial challenge before and after injections of allergen extract; (2) to obtain some preliminary data on the possibility that high titres of antibody in the serum during injection of large doses of antigen may favour formation of antigen-antibody complexes (to this end, serum complement levels were measured serially); and (3) the specificity of fluctuations in leucocyte sensitivity was examined by simultaneous determinations of sensitivity to the allergen given by injection and to another, unrelated allergen.

Subjects and treatment plan

Prospective study

Eight children, aged between 5 and 14 years, with sensitivity to alternaria, ragweed or dog epithelium extracts were selected from inpatients with severe asthma at National Jewish Hospital, for intensive treatment with a single extract over a 4-week period. Six of the eight subjects had been treated in the past with mixtures containing the allergens under study, and one patient (Subject 408) had not received any form of injection therapy. None of the patients had received injection therapy in the past 6 months. All of the eight children had required regular medication with theophylline and ephedrine orally, and four of them had needed alternate-day medication with prednisone orally to keep them relatively symptom-free. The children were closely supervised in the hospital during the period of intensive therapy, and all injections were given subcutaneously by the authors. Initially injections were given two to four times a day, in order to reach maintenance doses of 10,000 protein nitrogen units (PNU) in 6–17 days, depending on the tolerance of the individual patient. Five patients were treated with alternaria extracts, two with dog epithelium extracts, and one with a mixture of tall and short ragweed extracts in total doses of 201,735–515,520 PNU given over 4 weeks (Table 1).

Four of the eight patients were given continued treatment for a further 10–20 weeks, because these children were available for an extended study period.

The subjects were followed by frequent urinalysis and haematological examinations, and serum complement determinations.

Retrospective study

Twenty-three children and adolescents between 5 and 20 years of age with asthma and/or allergic rhinitis were selected for study from the University of Colorado Medical

Table 1. Data from prospective study: treatment and histamine release from leucocytes

Patient No.	Antigen	Weeks after beginning of treatment	Comparison of leucocyte sensitivity					Maximum histamine release		
			Cumulative dose (PNU)	Allergen concentration ($\mu\text{g PN/ml}$)	Per cent histamine release		Allergen concentration ($\mu\text{g PN/ml}$)	Per cent release	Allergen concentration ($\mu\text{g PN/ml}$)	Per cent release
					Initial	Final				
Intensive treatment	382 Alternaria*	4	505,520	0.003	36	69	0.1	81	0.01	71
	395 Alternaria*	4	515,520	0.03	41	83	1.0	90	0.3	84
	396 Alternaria*	4	501,520	0.001	37	72	0.01	73	0.01	83
	411 Alternaria*	4	201,735	0.003	40	75	0.03	61	0.03	80
	408 Alternaria†	4	204,335	0.003	63	64	0.1	94	0.03	71
	407 Ragweed*	4	209,445	0.0001	64	8	0.001	79	0.01	78
	403 Dog epithelium*	4	214,285	0.1	50	32	1.0	85	1.0	66
	435 Dog epithelium*	4	215,785	0.1	22	35	1.0	62	1.0	74
Intensive, followed by prolonged treatment	408 Alternaria†	14	249,935	0.003	63	55	0.1	94	0.03	75
	407 Ragweed*	15	257,445	0.001	79	2	0.001	79	0.01	22
	403 Dog epithelium*	24	394,285	0.1	50	1	1.0	85	10.0	15
	435 Dog epithelium*	24	315,785	0.1	22	36	1.0	62	1.0	63

* From Greer Laboratories, North Carolina.

† From Center Laboratories, New York.

‡ Final, divided by Initial, per cent histamine release. Values approaching 1.0 mean no change in leucocyte sensitivity. Values <1.0 = decreased sensitivity.

Center Pediatric Allergy Clinic. These children had already been treated on a perennial basis for a minimum of 14 months by injection every 2–4 weeks of mixtures containing either alternaria or tall and short ragweed allergens. No further therapy was given by us. The treatment mixtures contained between five and eighteen allergens, many of which were antigenically related to one another. Nine children were treated with mixtures containing alternaria extract; the total dose of alternaria allergen contained in the mixtures was 19,524–243,805 PNU, given over periods between 15 months and 7 years (Table 2). Fifteen children were treated with mixtures containing ragweed extract; the total dose of ragweed allergens contained in the mixtures was 13,180–184,270 PNU, given over periods between 14 months and 8 years (Table 3). All of the patients had at least a 5 mm weal response to intradermal testing with the allergen under study at a concentration of 1000 PNU/ml, before injections had been commenced.

Materials

Allergen extracts for treatment were obtained from Greer Laboratories, North Carolina, and Center Laboratories, New York, and used as indicated in Table 1. Dilutions from these extracts were prepared in phosphate buffered saline solution with phenol for skin testing and bronchial provocation. Lyophilized extracts for leucocyte histamine release studies were supplied by Greer and Center Laboratories. Stock solutions of extract were reconstituted from lyophilized material with the same lot number every 2 months, and sterile dilutions of these were made every 2 weeks. Patients treated with Greer Laboratories allergens were studied by *in vitro* methods with Greer allergens, and patients treated with Center Laboratories allergens were similarly studied with allergen from Center Laboratories.

Methods

Leucocyte histamine release

Leucocyte sensitivity to allergen was measured as described previously (May *et al.*, 1970a, b). This method employs normal serum in the tube containing leucocytes and allergen in order to provide optimum conditions for histamine release. A concentration–response curve was established with each specimen of leucocytes. Serial variations in leucocyte sensitivity were followed by comparing the percentages of the total leucocyte histamine content released by a given concentration of allergen (May *et al.*, 1970a, b).

Serum antigen-neutralizing capacity

An estimate of the capacity of serum to bind allergen *in vitro* was determined as described previously (May *et al.*, 1970b, 1972a), using three adult donors with leucocytes which released 45–50% of their histamine at 0.001–0.01 μ g protein nitrogen/ml (1 μ g protein nitrogen = 100 PNU) of alternaria, ragweed or dog extracts. Sera for antigen neutralization experiments were stored at -70°C .

Skin testing

Skin tests were done by intradermal injection of about 0.01 ml of allergen extracts containing 10, 100 and 1000 PNU/ml, each concentration being tested in triplicate in

Table 2. Patients treated with mixtures containing alternaria

Patient No.	Age (years)	Disease*	Duration of therapy (years)	Total dose alternaria (PNU)	Total dose alternaria in last year (PNU)	Maximum histamine release†	Antigen concentration‡ required for:			
							Maximum release	30% release		
390	16	2/12	A, SAR	6	0/12	61,956	29,166	78	0.1	<0.03
391	8	8/12	A	1	9/12	57,302	30,500	67	3	<0.03
393	15	0/12	A, PAR	7	7/12	243,805	31,500	30	10	10
397	13	3/12	A	1	3/12	19,524	18,750	58	0.1	<0.03
398	14	8/12	A, SAR	3	6/12	88,964	21,250	39	10	3
400	5	0/12	A	1	9/12	25,639	15,250	52	1	0.1
404	14	10/12	A, PAR	1	10/12	66,878	33,142	58	0.3	0.01
416	15	3/12	A	1	11/12	71,350	50,000	41	0.1	0.03
445	8	4/12	A	1	4/12	50,098	34,283	82	3	0.03

* A = asthma, SAR = seasonal allergic rhinitis, PAR = perennial allergic rhinitis.

† % total leucocyte histamine.

‡ µg protein nitrogen/ml.

Table 3. Patients treated with mixtures containing ragweed

Patient No.	Age (years)	Disease*	Duration of therapy (years)	Total dose ragweed (PNU)	Total dose ragweed in last year (PNU)	Maximum histamine release†	Antigen concentration‡ required for:	
							Maximum release	30% release
383	11 0/12	A	3 9/12	49,094	10,000	30	0.3	0.3
384	20 6/12	SAR	8 2/12	118,333	47,633	89	3	0.3
385	14 2/12	A, PAR	3 2/12	58,403	9,250	29	3	3
386	10 0/12	A	1 2/12	23,622	23,600	84	3	0.3
392	6 6/12	A	1 3/12	13,180	13,148	33	10	1
394	12 9/12	A, PAR	1 8/12	53,264	47,333	86	3	0.1
401	12 2/12	A, PAR	4 0/12	31,951	20,333	3	—	—
402	12 9/12	A, SAR	1 10/12	18,842	13,166	57	3	0.3
424	14 2/12	A, PAR	2 11/12	119,190	39,332	74	1	0.1
427	10 9/12	SAR	2 6/12	62,703	49,333	> 63	< 0.03	< 0.03
428	12 10/12	A	2 8/12	73,898	58,667	76	10	0.1
430	10 11/12	A	2 0/12	95,187	47,000	42	10	1
442	11 7/12	A	3 7/12	184,270	47,000	3	—	—
445	8 4/12	A	1 4/12	66,799	60,000	61	3	< 1
446	9 10/12	A	1 4/12	99,924	76,666	24	10	> 10

* A = asthma, SAR = seasonal allergic rhinitis, PAR = perennial allergic rhinitis.

† % total leucocyte histamine.

‡ µg protein nitrogen/ml.

initial and subsequent tests. All tests of each allergen were performed with serial dilutions from a stock allergen extract with the same lot number.

Bronchial challenge

Testing of sensitivity to inhalation of graduated doses of allergens in aqueous aerosol was performed in Dr Bronsky's laboratory as described by Bronsky & Ellis (1969). In each subject tested, minimum threshold doses of allergen needed to induce a fall in the peak expiratory flow rate were determined before injection treatment was commenced. These doses ranged between 50 and 2000 PNU. Testing of bronchial sensitivity to allergen after treatment was done in an identical manner.

Serum complement studies

Total haemolytic complement titres of serum in 50% haemolytic units (CH_{50}) were determined in Dr Kohler's laboratory by the method of Mayer (1961). Complement components of two sera were assayed by radial immunodiffusion by the method of Kohler & Müller-Eberhard (1967).

Results

In order to ensure that the histamine release method used in our laboratory was as sensitive as possible, we confirmed and extended the previously reported finding (May *et al.*, 1972a; Lichtenstein & Osler, 1966) of the enhancing effect of normal human serum on antigenic histamine release from the leucocytes of allergic subjects. Histamine release with serum was compared to release without serum and without human serum albumin, because albumin has been found to inhibit histamine release from leucocytes of some subjects (May *et al.*, 1972a). Antigenic histamine release was enhanced by 8% normal group AB human serum in all of the nineteen subjects studied (Table 4). In seven subjects leucocytes which were virtually unresponsive to an allergen in the absence of serum released easily measurable amounts of histamine when studied in a medium containing 8% serum. These findings supported our use of serum in the method for the determination of leucocyte sensitivity in the study of the incidence of leucocyte unresponsiveness to allergens.

Prospective study

All intensively treated patients showed immediate erythematous local reactions at the site of the subcutaneous injections ranging up to 35 mm in diameter, often followed by induration persisting for several hours. However, four of the patients often had minimal local reactions, including one (Subject 407) who usually had minimal or no local reactions. Some patients had diffuse swelling hours after an injection, but the size of local reactions was not used to determine the amount of subsequent doses of allergen. Four patients (Subjects 396, 382, 411 and 407) had a mild generalized reaction characterized by wheezing or urticaria. This was taken as an indication for reduction or omission of the following dose. Systemic anaphylaxis was not encountered.

In the eight asthmatic children who were given injections of alternaria, ragweed or dog epithelium extracts in a 4 week period of intensive therapy, leucocyte sensitivity was measured at 6 or 7 day intervals over the treatment period. Seven of the eight patients did not show a marked decrease in their leucocyte sensitivity to the extract with which they were treated during this initial 4 week period of high dosage (Table 1). There was no downward trend in the maximum leucocyte histamine release, nor was

Table 4. Effect of normal group AB human serum on antigenic release of histamine from leukocytes

Patient No.	Allergen extract	Concentration of allergen for maximum release ($\mu\text{g PN/ml}$)	Maximum histamine release (%)	
			Serum-free buffer	Buffer with 8% human serum
434	Dog epithelium	10.0	26	41
436	Alternaria	0.1	46	63
449	Alternaria	0.1	51	84
449	Ragweed	0.1	48	71
500	Timothy*	0.01	69	85
502	Dog epithelium	1.0	2	12
502	Timothy	0.1	43	69
505	Alternaria	0.1	53	72
505	Ragweed	1.0	3	16
509	Timothy	0.001	54	72
510	Ragweed	0.01	43	65
511	Alternaria	0.1	67	73
512	Ragweed	0.01	73	82
513	Alternaria	1.0	1	23
519	Ragweed	0.1	46	66
519	Timothy	0.01	39	56
520	Timothy	1.0	7	16
521	Alternaria	1.0	3	10
521	Dog epithelium	1.0	75	80
523	Alternaria	1.0	5	20
523	Ragweed	0.01	22	35
524	Alternaria	0.1	4	11
524	Ragweed	0.01	4	15
527	Timothy	0.01	64	73
531	Alternaria	0.1	14	27
533	Alternaria	0.01	18	56

* Timothy grass pollen.

there any significant alteration of the concentration of allergen at which maximum release was found. During the first 4 weeks of treatment, one patient (Subject 407) did show a progressively decreasing leucocyte sensitivity at low allergen concentrations only.

Intradermal tests with the allergen extract used in treatment were done in six children before, during and immediately after 4 weeks of intensive therapy. Although minimum skin test doses for positive responses were not established in three of the patients, there was no obvious change in the skin reactivity of any of the patients tested (Table 5). In addition, bronchial reactivity to the extract used in treatment did not change in the five children who could be tested (Table 6). Of the three children who could not be tested by bronchial provocation, one was too young to cooperate in the test, and in the other two the baseline pulmonary function was too unstable to allow reliable testing by this method.

Our failure to demonstrate significant changes in leucocyte sensitivity of most of the patients after 4 weeks of intensive therapy prompted continued treatment with the same allergens given at a more conventional rate in the four patients who remained

Table 5. Results of intradermal skin tests*

Patient No.	Before treatment			After 4 weeks intensive treatment		
	10 PNU/ml	100 PNU/ml	1000 PNU/ml	10 PNU/ml	100 PNU/ml	1000 PNU/ml
395	0	3.0	6.6	ND	4.0	5.7
403	0	4.7	7.7	ND	5.3	7.0
407	6.6	7.3	ND	4.0	5.0	5.0
408	5.7	6.3	8.0	4.3	4.7	5.3
411	5.0	6.7	8.3	4.3	4.7	5.0
435	0	2.7	5.0	ND	3.7	7.0

ND = Not done.

* Mean net diameter (mm) of weals in triplicate tests with allergen extract used in treatment; the diameter of any bleb persisting in a control injection of diluent has been subtracted.

Table 6. Threshold doses (PNU) for positive bronchial challenge*

Patient No.	Before treatment	After treatment
395	200	1000 (day 28)
395		200 (day 46)
396	200	200 (day 76)
403	100	25.5 (day 169)
407	50	50 (day 77)
407		12.5 (day 97)
435	2000	1000 (day 35)

* Dose at which significant reduction (> 15%) in peak expiratory flow rate was observed.

available for study. These patients were treated for total period of 14–24 weeks and were given total doses of allergen ranging between 249,935 and 394,285 PNU (Table 1). The patients treated with alternaria and one of the two patients treated with dog epithelium extract did not achieve a persistent reduction of leucocyte sensitivity (Table 1 and Figs. 1 and 2). The other patient treated with dog epithelium extract (Subject 403) did show a decline in leucocyte sensitivity after 16 weeks of therapy. The one patient, Subject 407, who was treated with ragweed extract, also showed a reduction of leucocyte sensitivity to ragweed extract beginning within 4 weeks, with maximal histamine release decreasing from 79% to 22% (Table 1 and Fig. 3). However, complete loss of leucocyte sensitivity to allergen was not achieved, nor was there a change in the allergen concentration required for maximal release.

In addition to serial determinations of leucocyte sensitivity to the allergen extract used for treatment, all patients were studied by simultaneous determinations of sensitivity to another unrelated allergen extract. The findings are reported more completely

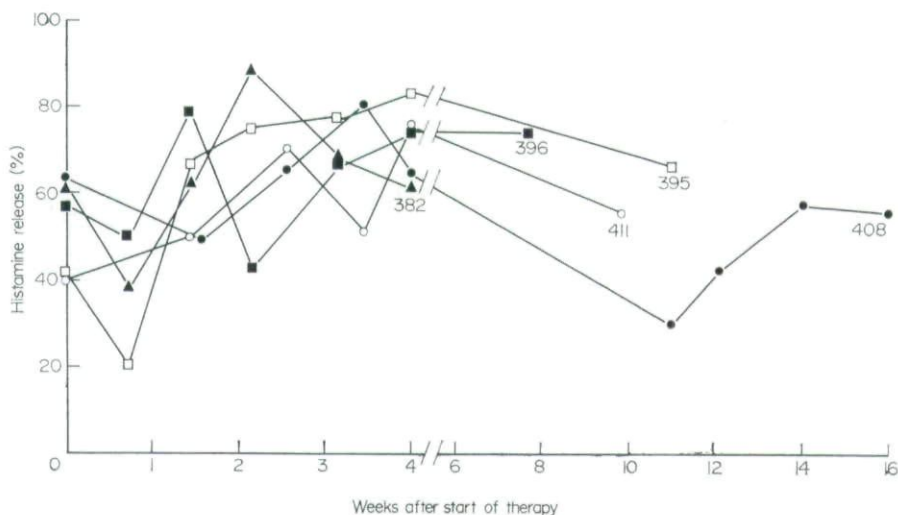


Fig. 1. Serial determinations of leucocyte sensitivity to alternaria (histamine released at given concentrations) in the subjects treated intensively with alternaria extract: ▲, Subject 382, 0.03 μ g protein nitrogen (PN) /ml; □, Subject 395, 0.03 μ g PN/ml; ■, Subject 396, 0.003 μ g PN/ml; ●, Subject 408, 0.003 μ g PN/ml; ○, Subject 411, 0.003 μ g PN/ml.

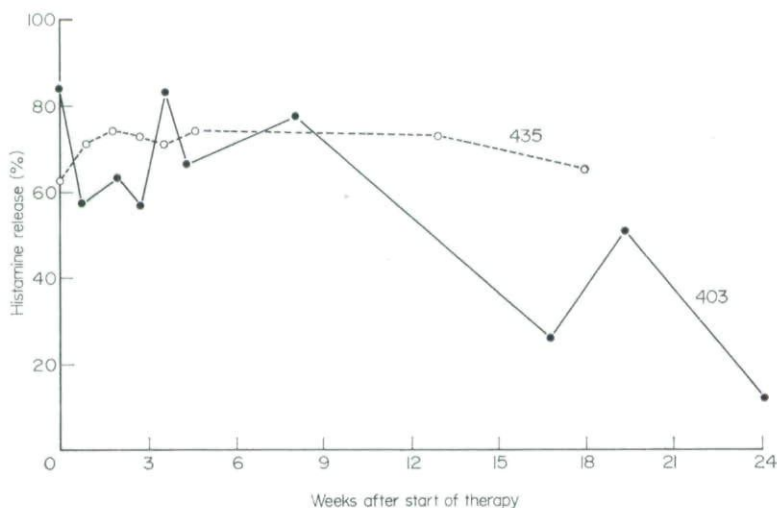


Fig. 2. Serial determinations of leucocyte sensitivity to dog epithelium (histamine released in response to 1 μ g PN/ml) in two patients treated intensively with dog epithelium extract.

in another paper (May, Schumacher & Williams, 1972b). Here it is most pertinent to note that in Subject 407 leucocyte sensitivity to alternaria decreased simultaneously and to the same degree as the reduction of sensitivity to ragweed (Fig. 3). The other patient who showed a marked reduction in leucocyte sensitivity when treated with dog epithelium extract (Subject 403) was not sufficiently sensitive to allergens other than animal dander extracts to determine fluctuations in sensitivity to an unrelated allergen.

None of the children given extended treatment following a period of intensive

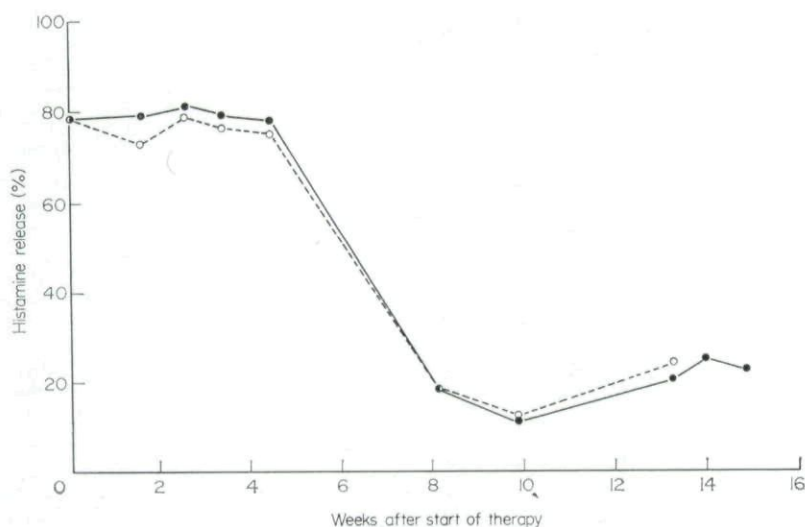


Fig. 3. Serial determinations of leucocyte sensitivity to ragweed and alternaria (histamine released in response to given concentrations) in a patient treated intensively with ragweed extract (Subject 407). ●, Ragweed extract 0.01 μ g PN/ml; ○, alternaria extract 0.1 μ g PN/ml.

Table 7. Changes in serum complement levels in patients given intensive followed by conventional therapy

	Day of therapy					
	0	7	13	25	117	135
SUBJECT 403						
Complement titre (CH_{50} /ml)	45	36	30	36	29	24
Cumulative dose dog extract (PNU)	0	4,785	64,285	184,285	314,285	344,285
Antigen neutralizing capacity	4	*	*	*	10	23
	Day of therapy					
	0	11	18	24	71	98
SUBJECT 408						
Complement titre (CH_{50} /ml)	35	38	26	22	23	32
C4	0.468			0.330		
C3	1.60			1.12		
Cumulative dose alternaria (PNU)	0	41,335	107,335	164,335		249,935
Antigen neutralizing capacity	4			120	100	

* Not possible to determine, because patient's serum released histamine from donor leucocytes in the absence of added allergen.

Table 8. Serum antigen neutralizing capacity

Patient No.	Treatment extract							
	Alternaria	Alternaria	Alternaria	Alternaria	Alternaria	Ragweed	Dog epithelium	Dog epithelium
ANC before intensive treatment	382	395	396	411	408	407	403	435
ANC during intensive treatment	7	4	22	7	4	5	4	27
ANC after intensive treatment	ND	79 (day 15) [†]	438 (day 12)	ND	120 (day 24)	2 (day 18)	*	321 (day 25)
	673 (day 29)	70 (day 28)	63 (day 28)	177 (day 55)	100 (day 71)	5 (day 31)	23 (day 135)	27 (day 126)
	148 (day 89)	288 (day 86)	40 (day 90)			17 (day 93)		

ND = Not done.

* Not possible to determine because patient's serum at this time released histamine from donor leucocytes, in the absence of added allergen.

[†] Day of injection therapy.

therapy showed any significant alteration in the size of the skin test responses to the treatment allergens. Moreover, sensitivity to the treatment allergens on bronchial challenge testing remained unchanged in the two patients who had shown reduced leucocyte sensitivity.

There was no clinical evidence of circulating antigen-antibody complex disease in any of the patients, unless delayed swelling at the site of injections should be viewed as such. In particular, there was no fever, polyarthralgia, lymphadenopathy, and no proteinuria or microscopic haematuria in urine specimens obtained three times a week during injections. However, there were small reductions in haemolytic complement levels of serum in three patients (Subjects 382, 396 and 411), and there were significant reductions in two patients (Subjects 403 and 408). A study of individual complement components in sera from Subject 408 revealed a reduction of C3 and C4 levels (Table 7).

The antigen neutralizing capacity (ANC) of serum obtained at intervals during the course of treatment was measured. Changes in serum complement levels were not associated with large rises in serum antibody levels (Tables 7 and 8). The patient with significant fall in his serum complement levels had a 30-fold increase in serum ANC. However, other patients who had similar rises in serum ANC did not show changes in serum complement levels. The patient who had a marked decrease in leucocyte sensitivity during therapy (Subject 407) had virtually no increase in serum ANC, and no change in the serum complement level. Of the group of eight patients, this patient had the lowest incidence of local reactions to injections. The other patient who showed significant reduction in leucocyte sensitivity (Subject 403) also had a poor serum ANC response.

Retrospective study

Of the nine children who had received mixtures containing alternaria extract, none were found to have leucocytes which were insensitive to alternaria *in vitro*. At least 30% histamine release was demonstrable in all patients and the maximum histamine release ranged up to 82% (Table 2).

Of the fifteen children who had been given mixtures containing ragweed extract, only two proved to have leucocytes which did not release a significant amount of histamine on exposure to ragweed *in vitro*, and the other thirteen patients released 24–89% of their total leucocyte histamine (Table 3). One of the two patients with unresponsive leucocytes (Subject 442), treated with a total of 184,000 PNU of ragweed extract over 3½ years, did not react to a bronchial provocation test with ragweed, even at a dose level of 10,000 PNU. However, an intradermal skin test with 1000 PNU/ml of ragweed produced a 6 mm weal. The other patient with leucocyte unresponsiveness to ragweed *in vitro* had been treated with a mixture containing a total of only 32,000 PNU of ragweed over a 4 year period. This patient had only a 5 mm weal response to an intradermal test with ragweed extract at 1000 PNU/ml before the start of treatment; it is possible that this patient's leucocytes may have been unresponsive even before the course of injection therapy.

Discussion

Our previous experience of rare occurrence of loss of leucocyte sensitivity (as measured by antigenic release of histamine) during injections of allergen extract in children (May *et al.*, 1972a) was confirmed by the present study. In the studies by Levy, Lichten-

stein and co-workers (Sadan *et al.*, 1969; Levy *et al.*, 1971) of preseasonal injection therapy of children with relatively small amounts of ragweed extract, it was claimed that loss of leucocyte sensitivity to ragweed antigen E occurred in three of eighteen treated children in their first study. In their second study complete loss of leucocyte sensitivity to ragweed antigen E was said to have occurred in four of twenty-four treated children. In contrast, we had previously found only one instance of complete loss of leucocyte sensitivity during injection of allergen extracts in forty-six children, using both customary and intensive dosage regimens either preseasonally or perennially for periods of up to 4 years (May *et al.*, 1972a).

Leucocyte unresponsiveness did not develop in any of the eight children studied prospectively in the present study. Our finding of two instances of leucocyte unresponsiveness in the group of twenty-four children whose leucocyte sensitivity was determined retrospectively after prolonged courses of customary injection therapy means only that unresponsiveness is not a common end result of such therapy; the two found to have insensitive leucocytes may not have had sensitive leucocytes before therapy.

The occurrence of leucocyte unresponsiveness in the present study was as rare as in our earlier study (May *et al.*, 1972a) of entirely different groups of children. Therefore, our previously expressed suspicion (May *et al.*, 1972a) is increased, that the explanation for the claim of Lichtenstein and co-workers of a higher incidence of unresponsiveness in their studies (Sadan *et al.*, 1969; Levy *et al.*, 1971), is based on their use of a suboptimal system for measurement of leucocyte sensitivity. They did not include 8% normal human serum in the mixture of leucocytes and antigen during the histamine release procedure. The serum results in enhancement of histamine release by 20–80% (May *et al.*, 1972a). Leucocytes which release low but easily measurable amounts of histamine in the presence of serum could appear to be unresponsive if serum were not included. To learn the true incidence of unresponsiveness, serum must be employed.

Although we were not able to induce complete loss of leucocyte sensitivity with an intensive course of allergen injections, we did observe a marked reduction in leucocyte sensitivity in two of four patients who were given prolonged conventional dose therapy after an initial 4 week period of intensive treatment. None of the intensively treated patients, including the two who showed reduction in leucocyte sensitivity, showed any alteration in the skin test responses. This finding is supported by the previous study (May *et al.*, 1972a), and differs from the original report by Freeman (1930) of rapid injection therapy associated with a large decrease in skin test responses.

Of particular interest is the observation that under the conditions of the present study, marked reductions in leucocyte sensitivity in asthmatic patients are not necessarily associated with any decrease in bronchial sensitivity to allergen. It therefore seems appropriate to question the relationship between blood leucocyte sensitivity and bronchial reactivity to allergen, and to consider the possibility that even complete desensitization of blood leucocytes may not suffice to achieve reduction or loss of responsiveness to the inhalation of allergenic material. In a placebo-controlled study, Aas (1971) reported that injection treatment of children with house dust extracts was associated with a decrease in bronchial reactivity. Since treatment in his study was continued for 2½–3 years, lack of changes in bronchial reactivity following intensive therapy in the present study may be related to the limited duration of the treatment.

In one of the two children who showed marked reduction in leucocyte sensitivity to allergen, we observed a simultaneous decrease in sensitivity to another unrelated

allergen (with which he was not treated), indicating that the effect of injection treatment in this patient was not specific for the allergen employed. This finding is consistent with the previous report of a patient with loss of leucocyte sensitivity to two allergens during therapy with only one of them (May & Aduna, 1971), and is the subject of another paper (May *et al.*, 1972b).

Most of the intensively treated patients had rises in serum antigen neutralizing capacity similar to those observed in the previous study (May *et al.*, 1972a). It is of interest that reduction in leucocyte sensitivity was not related to the degree of increase in serum ANC since the children (Subjects 403 and 407) with a significant decline in leucocyte sensitivity had the poorest serum ANC responses. The lack of correlation between leucocyte sensitivity and serum ANC in injection therapy is consistent with data reported by Levy *et al.* (1971). In Subject 403 it was not possible to assess serum ANC during the period of intensive treatment because in control incubations his serum released histamine from the cells of two different dog-sensitivity patients without the addition of allergen. One possible explanation would be the presence of free antigen (from dog epithelium extract injected) in his circulation at the time serum was collected.

Our finding of a decrease in serum haemolytic complement levels in two of the intensively treated patients suggests that circulating antigen-antibody complexes may have been formed in these patients. Although there were no clinical findings of disease due to complex formation, alteration of serum complement levels represents a possibly adverse effect of injection therapy which had not received the attention it deserves.

References

- AAS, K. (1971) Hyposensitization in house dust allergy asthma. A double-blind controlled study with the evaluation of the effect on bronchial sensitivity to house dust. *Acta Paediatrica Scandinavica*, **60**, 264.
- BRONSKY, E.A. & ELLIS, E.F. (1969) Inhalation bronchial challenge testing in asthmatic children. *Pediatric Clinics of North America*, **16**, 85.
- FREEMAN, J. (1930) "Rush" inoculation, with special reference to hay-fever treatment. *Lancet*, **i**, 744.
- KOHLER, P.F. & MÜLLER-EBERHARD, H.J. (1967) Immunochemical quantitation of the third, fourth and fifth components of human complement: Concentrations in the serum of healthy adults. *Journal of Immunology*, **99**, 1211.
- LEVY, D.A., LICHTENSTEIN, L.M., GOLDSTEIN, E.O. & ISHIZAKA, K. (1971) Immunologic and cellular changes accompanying the therapy of pollen allergy. *Journal of Clinical Investigation*, **50**, 360.
- LICHTENSTEIN, L.M. & OSLER, A.G. (1966) Studies on the mechanisms of hypersensitivity phenomena. XI. The effect on the release of histamine from human leukocytes by ragweed pollen antigen. *Journal of Immunology*, **96**, 159.
- MAY, C.D. & ADUNA, N. (1971) Loss and recovery of leukocyte sensitivity to antigenic histamine release during intensive injection therapy with allergen extract. *Journal of Allergy and Clinical Immunology*, **48**, 313.
- MAY, C.D., LYMAN, M., ALBERTO, R. & ADUNA, N. (1972a) Immunochemical effects of injection therapy with allergens by customary and intensive dosage regimens in children. *Pediatrics*, **49**, 536.
- MAY, C.D., LYMAN, M., ALBERTO, R. & CHENG, J. (1970a) Procedures for immunochemical study of histamine release from leukocytes with small volume of blood. *Journal of Allergy*, **46**, 12.
- MAY, C.D., LYMAN, M., ALBERTO, R. & CHENG, J. (1970b) Immunochemical evaluation of antigenicity of house dust extract. *Journal of Allergy*, **46**, 73.
- MAY, C.D., SCHUMACHER, M.J. & WILLIAMS, C.S. (1972b) Significance of concordant fluctuations and loss of leukocyte sensitivity to two allergens during injection therapy with one: non-specific desensitization. *Journal of Allergy*, **50**, 99.

- MAYER, M.M. (1961) Complement and complement fixation. In: *Experimental Immunochemistry* (Ed. by E. A. Kabat), 2nd edn, p. 133. Charles C. Thomas, Springfield, Illinois.
- SADAN, N., RHYNE, M.B., MELLITS, E.D., GOLDSTEIN, E.O., LEVY, D.A. & LICHTENSTEIN, L.M. (1969) Immunotherapy of pollinosis in children. Investigation of the immunologic basis of clinical improvement. *New England Journal of Medicine*, **280**, 623.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.