

Further studies of concordant fluctuation in sensitivity of peripheral leucocytes to unrelated allergens and the meaning of nonspecific 'desensitization'

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Summary

These studies pertain to the significance and mechanism of concordant fluctuation in sensitivity of peripheral leucocytes to release of histamine by unrelated allergens.

One type of concordant fluctuation occurs during injection of allergen. In this type, reduction of leucocyte sensitivity is only partial and recovery is rapid when injection of allergen ceases, and the reduction is related to degranulation of basophils and decrease in the histamine content. The nonspecific nature of the concordant, proportionate reduction in sensitivity to unrelated allergens suggests degranulation of a population of basophils in the circulation that apparently carries the reaginic antibodies for all allergens to which the subject is hypersensitive.

Another type of concordant loss of peripheral leucocyte sensitivity to unrelated allergens occurs uncommonly, without clear relation to allergen injection. In this type, reduction of leucocyte sensitivity proceeds gradually to the point of unresponsiveness to allergen and persists for prolonged periods. The nonspecific nature of this type is not associated with decrease in histamine content of the peripheral leucocytes (basophils).

In both types, simultaneous determination of other indices of hypersensitivity (sensitivity of skin and bronchi, and reaginic antibody content of serum) showed no significant changes concomitant with fluctuation in peripheral leucocyte sensitivity. This suggests basophils in the peripheral blood behave differently from mast cells in tissues.

The findings point to important limitations in the use of *in-vitro* measurement of sensitivity of peripheral leucocytes (basophils) as an index of hypersensitivity of a person and as a means of monitoring the efficacy of injection therapy. The term 'desensitization' should be used precisely to distinguish loss of hypersensitivity of tissues throughout the body from reduced sensitivity of only certain cells.

Introduction

Our original observation of concordant decrease in sensitivity of peripheral leucocytes

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of allergic subjects to release of histamine by two unrelated allergens during injection therapy with one of the allergens were included in a case report in 1971 (May & Aduna, 1971). The next year, a preliminary report set forth our initial experiments to elucidate the mechanism of this phenomenon (May, Schumacher & Williams, 1972a).

The present paper provides the results of more extensive studies on the manner whereby this sort of non-specific 'desensitization' of peripheral leucocytes may occur during injection of an allergen into a hypersensitive subject. In addition to further data on fluctuations of leucocyte sensitivity to two unrelated allergens during intravenous or subcutaneous injection of one of the allergens, results are presented from simultaneous measurement of histamine content of blood leucocytes (basophils). Along with measurements of fluctuations in peripheral leucocyte sensitivity, other indices of hypersensitivity were followed, namely, sensitivity of skin and bronchi and reaginic antibody content of serum.

Besides the brief unsustained reduction in peripheral leucocyte sensitivity induced during injection of allergen, three instances of prolonged complete (also concordant) loss of peripheral leucocyte sensitivity to two unrelated allergens were observed while no allergen was being injected. The circumstances surrounding development of this unresponsiveness are presented.

Procedure and materials

Subjects

The studies were conducted with allergic children, 8–13 years of age, in residence for prolonged care in the National Jewish Hospital and Research Center.

The children were all prone to frequent severe attacks of asthma, and required average to high dosage of theophylline daily, as well as prednisone every other day to be nearly free of symptoms. The amounts of drugs given were found by experiment not to interfere with measurements on blood samples taken 12 hr after the preceding dose of theophylline and 48 hr after the preceding dose of prednisone, as was done in the present investigations. Their leucocytes released at least 50% of the histamine content with the allergen extracts employed in the observations at the beginning of the studies. Intracutaneous injection of the allergens under study elicited definite weal and flare responses. Most of the children had never received injections of allergen extracts, and the few who had been given injections had not received any for a year or more before the present study.

All procedures were carried out with the consent of fully informed parents and under the immediate supervision of the investigators. The protocol for intravenous infusion of allergen extracts was approved by the Committees on Human Experimentation of the National Jewish Hospital and the University of Colorado Medical Center.

Materials

Allergen extracts were obtained from Greer Laboratories, Lenoir, North Carolina. Concentration is expressed in PNU = Protein Nitrogen Units (1 PNU = 0.01 µg protein nitrogen). Stock solutions in buffer containing phenol were used in subcutaneous and intravenous injections, bronchial provocation tests, skin tests, and the Prausnitz-Kustner procedure. Dilutions for subcutaneous injection were made with buffer

containing phenol and for intravenous infusion and bronchial inhalation with 0.9% sodium chloride solution.

Stock solutions were of the same Lot Number. Dilutions were prepared freshly every 2 weeks and stored at 5°C.

Allergen extracts used in *in-vitro* determination of release of histamine from leucocytes were freeze-dried products free of preservative, prepared by Greer Laboratories. These were dissolved in sterile tris buffer of the composition used in the studies of leucocyte histamine release.

Methods

Antigenic release of histamine from leucocytes and antigen neutralizing capacity of serum were determined by methods previously described (May *et al.*, 1970a, b). Concentration-response curves were determined with four to six concentrations for each of two unrelated allergens at every measurement of leucocyte sensitivity.

Fluctuations in leucocyte sensitivity are detected by serial comparisons of percentage of leucocyte histamine released by a fixed concentration of allergen. The fixed concentration is selected from the serial determinations of histamine release with four to six concentrations of the two unrelated allergens.

Antigen-neutralizing capacity of the serum is measured by incubation of allergen with the subject's serum to permit binding of allergen by specific antibodies in the serum. Unbound allergen is detected by allowing sensitized leucocytes to react with the incubated serum-allergen mixture and measurement of histamine released. The amount of allergen bound or neutralized by the subject's serum is expressed as a number indicating how many times more allergen is bound by this subject's serum than by a control serum from a non-allergic person, e.g. ANC 10 = 10 times as much antigen (allergen) neutralized. For further elaboration, see previous reports (May *et al.*, 1970a, b).

Calculation of histamine content of the blood was made possible through measurement of histamine content of the leucocyte suspension prepared for study of *in-vitro* antigenic histamine release and knowledge of the amount of blood from which the leucocytes were obtained.

Basophils in the blood were counted by the method of Cooper & Cruickshank (1966).

Skin tests were performed with disposable syringes and needles, about 0.01 ml extract being injected intradermally. Concentrations injected are given in the Tables and Figures in Results. The results are expressed as net diameters of weal or flare; the diameter of any bleb persisting at the site of the control injection of diluent has been subtracted.

Estimation of reaginic antibody titre in the serum was performed by the Prausnitz-Kustner procedure in conventional manner (Sherman, 1968); undiluted serum and 1:10, 1:100 and 1:1000 dilutions were tested.

Appraisals of bronchial sensitivity to inhaled allergen were done through the kindness of Dr Edwin Bronsky in his laboratory at the National Jewish Hospital, according to a published procedure (Bronsky & Ellis, 1969). A significant response is a reduction greater than 20% in the Forced Expiratory Volume in 1 sec within 15 min. A significant change in sensitivity would be an increment of two dilutions in concentrations of allergen solution inhaled, as described in the published procedure.

Procedure for intravenous infusion of allergen extracts

Solutions of allergen extract were administered through ordinary equipment for continuous intravenous infusion except that instead of gravity, a mechanical infusion pump was used to control the rate (Harvard Instrument Co., Millis, Massachusetts; the pump could be set to deliver 1.8–50 ml/hr). Intravenous infusion was begun with a concentration of allergen of 1 PNU/ml at a rate of 1.8 ml/hr. This never caused any symptoms even in the highly sensitive children. The PNU/hr being infused was doubled at 30–60 min intervals until urticaria or increased wheezing occurred. The rate of infusion was then kept constant or reduced slightly until symptoms disappeared spontaneously, or occasionally 0.2 ml 1:1000 epinephrine s.c. or 25 mg diphenhydramine were given to hasten relief which usually came within a half hour. Subsequently, increases in rate of infusion could be made progressively until the maximum tolerable dosage was reached. As increased rates were tolerated, the concentration of allergen solution infused was increased. The infusion was given continuously for 72 hr, or 48 hr in a few cases. We were interested in studying the effects of the largest total PNU of allergen we could infuse without serious symptoms, but also in some cases in the least total PNU of allergen which would cause substantial reduction in leucocyte sensitivity. Therefore the maximum rates of infusion varied from 72 to 7000 PNU/hr and the total amount infused from 2500 to 180,000 PNU within 72 hr (details are present in the Tables of Results). By observing careful technique and close surveillance of the subjects, no serious reactions to intravenous infusion of allergen were experienced, although urticaria or slight increase in wheezing were observed at some time in the course of infusion in every subject. In our early experience, blood pressure was monitored frequently but as no effects were found, this practice was deemed unnecessary. Frequent examinations of the urine and blood urea nitrogen revealed no abnormalities.

Nevertheless, we wish to emphasize that intravenous infusion of allergen extract is not recommended for usual treatment.

Results

Typical concordant decrease and recovery in sensitivity of leucocytes to release of histamine by unrelated allergens during and after intravenous injection of one, is depicted in Figs. 1–3 in the first few days of the observations. Within 48–72 hr of continuous infusion of cautiously increased amounts of one allergen, sensitivity of leucocytes is decreased proportionately to two allergens by 30–100%. Upon cessation of infusion of allergen, sensitivity of leucocytes to both allergens regularly begins to recover concordantly within hours and may attain the level before infusion in 24 hr. There was no relation to any drugs the subjects may have received. The other measurements made during the courses of the subjects presented in Figs. 1–3 will be discussed later.

Our total experience with intravenous infusion of allergen into hypersensitive children includes four courses in three subjects in the preliminary report (May *et al.*, 1972a) and twenty-seven courses in twelve subjects in the present report. With the careful technique of intravenous infusion described in the section on Procedures, there were no serious reactions even though all the subjects were prone to severe asthma and usually exhibited marked sensitivity to the allergen infused. However, every subject, at some time during infusion of allergen, developed mild urticaria or wheezing, often

Table 1

Column 1*	2	3	4	5	6	7	8						
Allergens	Leucocyte sensitivity												
	Amount i.v./time (PNU)/(hr)	Max. rate (PNU/hr)	Conc. allergen (PNU/ml)	Histamine release			Histamine in blood						
				I (%)	F (%)	Δ (%)	I (ng/ml) (basoph./mm ³)	F (%)	Δ (%)				
										ANC	Symptoms	Conc. allergen for 50% hist. release before any infusions (PNU/ml)	
1. Subject 543—Period I													
A. Alternaria	75,000/72	1800	1	86	56	-35	0.7	28	17	-40		Urticaria	0.2
B. Timothy	none		100	78	39	-50							6
543—Period II													
A. Timothy	172,000/72	7000	100	77	23	-70	1.9	34	30	-12	1	Wheezing	
B. Alternaria	none		1	88	56	-36							
2. Subject 544—Period I													
A. Alternaria	53,000/72	1800	3	71	27	-62	2.3	30	17	-44	6	Wheezing	0.8
B. Ragweed	none		1000	56	41	-27							900
544—Period II													
A. Ragweed	145,000/72	5000	300	38	20	-46	1.7	72	47	-35	1	Wheezing	
B. Alternaria	none		0.1	77	56	-27							
3. Subject 567—Period I													
A. Ragweed	5,400/72	144	0.3	71	0	-100	1.1	53	12	-77		Urticaria	0.1
B. Timothy	none		0.3	65	7	-89		(59)	(33)			Wheezing	0.2
567—Period II													
A. Timothy	6,700/72	250	0.3	61	7	-88	1.1	25	25	0	1	Urticaria	
B. Ragweed	none		0.1	49	9	-81						Wheezing	
4. Subject 568													
A. Alternaria	2,500/72	72	0.3	76	46	-30	0.4	52	35	-33	9	Urticaria	0.1
B. Ragweed	none		30	64	14	-78		(42)	(19)			Wheezing	20
5. Subject 566													
A. Alternaria	33,500/48	1250	0.3	77	0	-100	1.2	34	9	-74	2	Urticaria	0.1
B. Ragweed	none		100	88	14	-84						Wheezing	10
6. Subject 545—Period I													
A. Timothy	180,000/72	7000	100	49	29	-41	1.9	23	9	-61		Wheezing	10
B. Alternaria	none		3	76	60	-21							1
545—Period II													
A. Alternaria	110,000/72	3000	3	82	17	-80	0.8	19	6	-69	3	Urticaria	
B. Timothy	none		100	57	0	-100		(25)	(2)				

Conc. allergen
for 50% hist.
release before
any infusions
(PNU/ml)

I F Δ
(ng/ml)
(basoph./mm³)

I F Δ
(%)

I F Δ
(%)

I F Δ
(%)

I F Δ
(%)

I F Δ
(%)

I F Δ
(%)

Table 1 (continued)

Column 1*	2	3	Leucocyte sensitivity								7	8	
Allergens	Amount i.v./time (PNU)/(hr)	Max. rate (PNU/hr)	Conc. allergen (PNU/ml)	Histamine release				Histamine in blood			ANC	Symptoms any infusions (PNU/ml)	Conc. allergen for 50% hist. release before
				I (%)	F (%)	Δ (%)	Ratio (%)	I (basoph./mm ³)	F (%)	Δ (%)			
13. Subject 569													
A. Timothy	11,800/72	400	1	67	9	-87	1.4	35	31	-12	1	Urticaria	0.3
B. Alternaria	none		1	49	20	-60		(58)	(28)			Wheezing	1
14. Subject 571													
A. Ragweed	14,200/72	500	10	63	13	-79	1.2	60	57	-5	6	Wheezing	3
B. Alternaria	none		3	66	24	-64		(61)	(34)				1
15. Subject 547													
A. Ragweed	9,700/48	700	1000	49	31	-37	1.6	20	10	-50	4	Urticaria	1000
B. Alternaria	none		30	65	50	-23						Wheezing	20

* Explanatory notes

Column 1. Allergens: A, injected intravenously; B, not injected but studied simultaneously.

Column 2. Total allergen injected in hours indicated. PNU = Protein Nitrogen Unit = 0.01 μ g Protein Nitrogen.

Column 3. Maximum rate of infusion of allergen attainable by end of infusion without induction of more than minor symptoms.

Column 4. Leucocyte sensitivity: Per cent histamine released by fixed concentrations of the allergens, initially (I) and at end of infusion (F) of the one allergen. The change in sensitivity (Δ) is expressed as a percentage [(I - F/I) $\times$ 100]. The ratio of changes in sensitivity to the allergens infused and not infused ($\Delta A/\Delta B$) indicates the degree of concordance in the changes; a ratio of 1.00 would result when the same change in leucocyte sensitivity occurs with both allergens.

Column 5. Calculation from histamine content of the leucocyte suspension which is prepared and analysed in procedure for histamine release from leucocytes, and the known volume of blood from which leucocytes were obtained. Numbers in Column 5 in parentheses are basophils/mm³.

Column 6. ANC = Antigen Neutralizing Capacity of serum.

Column 7. 'Wheezing' means more than usual.

Column 8. For each allergen, the original relative sensitivity of subjects in this series is indicated by the concentration of allergen required for 50% histamine release from their leucocytes before injections. As the biological equivalence of PNU is unknown, the sensitivity to the two unrelated allergens cannot be compared, nor can more than a rough comparison be made with results obtained with any other allergen extracts anyone else may be accustomed to using.

requiring no treatment or at most an occasional dose of epinephrine or diphenhydramine. However, intravenous injection is not recommended for ordinary treatment.

Data from twenty-three courses in twelve subjects regarding amount and rate of allergen infusion and the extent of concordant decrease in leucocyte sensitivity are given along with other relevant information in Tables 1 and 3.

Detailed consideration of Table 1, which includes explanatory notes and definitions of terms, reveals the following. Findings with the allergen infused and an unrelated allergen in each course of infusion are shown. In six subjects two courses are presented, one for infusion of each of the pair of allergens. The amount and rate of infusion of allergen was determined in part by our desire to learn the greatest that could be tolerated or to find the least which would cause considerable reduction in leucocyte sensitivity, and in part by the occurrence of any symptoms during infusion. Symptoms listed in Column 7 were frequent but minor, and the infusion was progressively adjusted to a rate just below that which was found to produce symptoms. The maximum rate achieved (Column 3) ranged from 72 to 7000 PNU/hr. The total amount infused in 48–72 hr (Column 2) ranged from 2500 to 180,000 PNU. An indication of the sensitivity of the leucocytes before any infusions were given is provided by the concentration of allergen required to release 50% of their histamine content (Column 8), ranging from 0.02 to 1000 PNU/ml. Leucocyte sensitivity (Column 4) was reduced 20–100% by the amounts and rates of infused allergen shown (Columns 2 and 3). The ratio of the percentage reduction of sensitivity by each of the unrelated allergens (Column 4) was usually about 1, indicating a proportionate reduction in leucocyte sensitivity to the allergen not infused and the one infused. In no case did infusion of allergen result in a significant increase in leucocyte sensitivity, either in the course of the infusion or in periods of observation afterwards extending to 15 weeks.

The histamine content of the leucocytes (basophils) (Column 5) declined during infusions of allergen in every case except one course in Subject 567, and even this subject exhibited urticaria during the infusion; the reduction ranged from 5 to 76% but not necessarily commensurate with the decrease in leucocyte sensitivity (Column 4). The total leucocyte count varied no more than usual. In a few cases the number of basophils in the peripheral blood were counted, and these revealed corresponding decreases during the infusions (Column 5, numbers in parentheses); more counts were not done because the direct measurement of histamine content in the leucocytes is more accurate and reflects the number of basophils in the blood.

In Fig. 1 are shown the results of measurement of histamine excretion in the urine during allergen infusion, in the one subject who could be studied in this manner after the method was established in our laboratory. Note that as histamine content of leucocytes decreased, urinary excretion increased. Upon cessation of infusion of allergen, the histamine content of leucocytes was quickly restored while the low urinary excretion suggests increased retention of endogenous histamine.

The antigen neutralizing capacity of the serum (ANC, Column 6) was minimal in all subjects before the initial courses of infusions as expected, because no previous injections of allergens had been given. The antibody response responsible for the ANC (IgG) does not cause increase in ANC until 7–10 days after injection of allergen. In cases where a second course of infusion was given 10 days or more after the first (Subject 522 in Table 1, and Subject 566 in Table 3) and a moderate elevation of ANC existed at the beginning of the second infusion, the leucocyte sensitivity was decreased

concordantly to the two allergens as readily as in the face of minimal ANC of the serum.

The intravenous infusion of allergen in the presence of appreciable specific IgG antibody in the serum conceivably could produce injurious antigen-antibody complexes. No characteristic symptoms one may expect were observed and numerous examinations of the urine and blood urea nitrogen gave no indication of renal damage during or after the infusions.

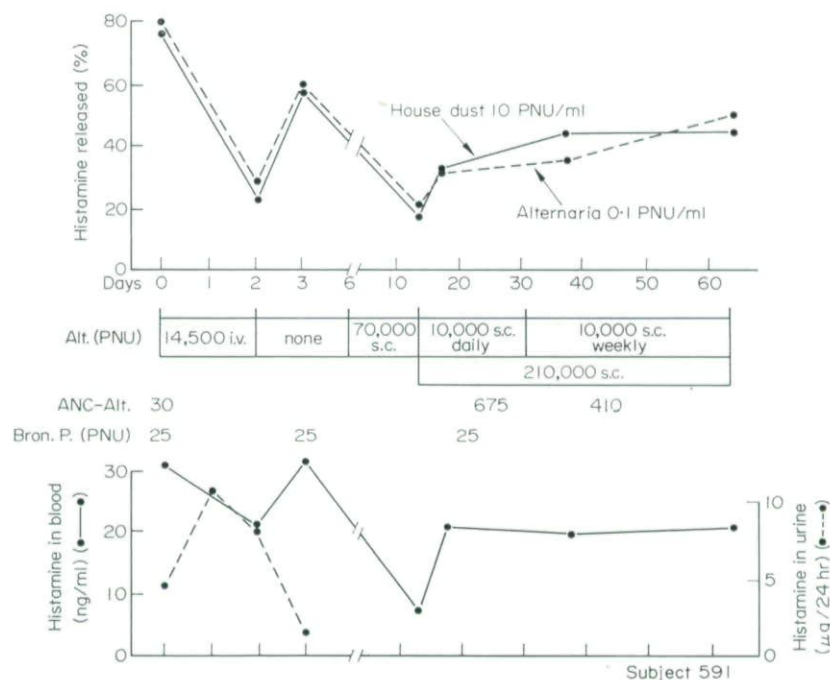


Fig. 1. Course of leucocyte sensitivity to histamine release by fixed concentrations of alternaria and house dust compared to level of histamine in blood cells and urinary excretion of histamine during and after intravenous infusion of alternaria, and subsequent subcutaneous injection of alternaria by an intensive dosage regimen. Results of antigen neutralizing capacity of serum (explained in Methods) and bronchial provocation with alternaria (lowest concentration in PNU required to give significant response) are also shown. i.v. = intravenous; s.c. = subcutaneous.

In three subjects, in whom intravenous infusion of one allergen caused the usual concordant reduction in leucocyte sensitivity to that one and an unrelated allergen, infusion of the other of the pair did not result in reduction of leucocyte sensitivity to either allergen (data not presented). Review of these revealed that leucocyte sensitivity was relatively low to the allergen which upon infusion did not decrease leucocyte sensitivity, and the amount and rate of allergen infusion were apparently inadequate to raise the concentration in the circulation to the level required to release histamine from the leucocytes (basophils). This supposition was supported by the lack of urticaria or wheezing during the infusions and the finding that the histamine content of the leucocytes was not decreased by the infusion.

Tables 2 and 3 and Figs. 1-3 contain data pertinent to the effect of considerable reduction in sensitivity of peripheral leucocytes by intravenous infusion of allergen on

Table 2. Effect of injection of an allergen on responses to allergens in skin test, Prausnitz-Kustner procedure and bronchial provocation

Column 1*	2	3	4	5	6	7	8
Allergens	Day	Amount injected (PNU)	Leucocyte sens. Δ (%)	ANC	Skin tests Conc. W/E (PNU/ml) (mm)	Prausnitz-Kustner procedure Serum dilution W/E (mm)	Bronchial provocation Conc. (PNU/ml) Result
Subject 515							
Alternaria	0			10	100 4/10	1:100 5/22	100 +
Ragweed	0				100 4/4		100 +
Ragweed	1-3	6,400 i.v.					
Alternaria	5-7	17,700 i.v.					
Alternaria	22-24	33,900 i.v.					
Alternaria	24		-98	100	100 4/10	1:100 12/30	Unstable
Ragweed	24		-88	100	100 3/4		Unstable
Subject 543							
Alternaria	0				100 7/30		100 +
Timothy	0			1	100 3/7		5000 -
Alternaria	1-3	74,700 i.v.					
Alternaria	6		0		100 8/22		100 +
Timothy	8-10	171,800 i.v.					
Timothy	10		-70	1	100 5/6		5000 -
Subject 567							
Ragweed	0				100 10/30	1:10 12/28	
Timothy	0			1	100 8/25	1:10 13/30	
Ragweed	1-3	5,400 i.v.					
Ragweed	3		-100		100 8/20		
Timothy	3		-95		100 8/20		
Ragweed	7		-9		100 8/20		
Timothy	7		-6		100 8/25		
Timothy	8-10	6,700 i.v.					
Ragweed	10		-81		100 8/25	1:10 15/30	
Timothy	10		-89	1	100 7/18	1:10 8/18	
Timothy	10-13	42,200 s.c.					
Timothy	14		-12				
Ragweed	14		+11				

Table 2 (continued)

Column 1*	2	3	4	5	6	7	8
Allergens	Day	Amount injected (PNU)	Leucocyte sens. Δ (%)	ANC	Skin tests Conc. W/E (PNU/ml) (mm)	Prausnitz-Kustner procedure Serum dilution W/E (mm)	Bronchial provocation Conc. (PNU/ml) Result
Subject 569							
Timothy	0			1	100 9/30		
Alternaria	0				100 6/20		
Timothy	1-3	11,800 i.v.					
Timothy	3		-60	1	100 10/25		
Alternaria	3		-87		100 6/20		
Subject 545							
Timothy	0				100 4/18	Undilu.	
Alternaria	0			5	100 7/30	1:10	5000 1000
Timothy	1-3	180,000 i.v.					
Timothy	3		-41				
Alternaria	3		-21				
Timothy	6		+12			Undilu.	
Alternaria	6		+10		100 8/25	1:10	6/18 7/25
Alternaria	7-9	110,000 i.v.					
Timothy	9		-100			Undilu.	
Alternaria	9		-78	2	100 7/20	1:10	10/25 10/32
Alternaria	10-20	120,000 s.c.					5000 500
Timothy	14		+28				
Alternaria	14		+5				
Timothy	41		+35			Undilu.	
Alternaria	41		+25	140		1:10	8/20 15/20
							1000 +

* Explanatory footnotes. See also data in Figs. 1-3 and Tables 1 and 3.

Column 3. PNU = Protein Nitrogen Unit = 0.01 μ g Protein Nitrogen.

Column 4. Leucocyte sensitivity: Change (Δ) in percentage histamine released by a fixed concentration of allergen, being a comparison between per cent released on Day 0 and on the successive days indicated.

Column 5. Antigen neutralizing capacity of serum.

Columns 6 and 7. Net diameters of weal/erythema; corrected for any bleb or erythema persisting at the sites of controls of diluent or serum. Results shown are with highest serial dilution which elicited definite reactions.

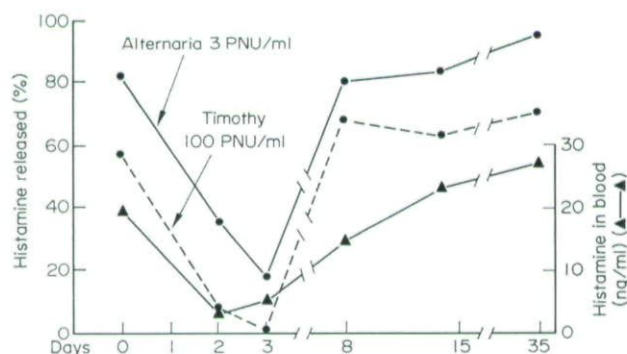
Column 8. Lowest concentration of allergen in procedure required to cause greater than 20% reduction in FEV₁ within 15 min. A significant change in sensitivity (defined in Methods) did not occur in any of the subjects.

other indices of hypersensitivity. In the five subjects in Table 2, intravenous infusions of allergen (given at times indicated in Column 2) caused 40–100% concordant reduction in leucocyte sensitivity (Column 4), but the responses to the allergens upon intracutaneous injection (Column 6) and bronchial inhalation (Column 8) were not significantly reduced, nor were the titres of specific reaginic antibody in the serum as determined by the Prausnitz-Kustner procedure (Column 7). In Cases 515 and 545 in Table 2, specific ANC of serum was considerably increased as expected 2 weeks or more after injection of allergen, without affecting the findings just described. Similar data from four additional subjects may be seen in Table 3 and Figs. 1 and 3 which revealed the same lack of significant decrease in other measures of hypersensitivity of the subject when sensitivity of leucocytes was markedly reduced. Table 4 summarizes the results of measurement of skin tests, reaginic antibody titre in serum (P.K. tests), and bronchial provocation in cases in which over 80% concordant reduction in leucocyte sensitivity was achieved for both the allergen infused and an unrelated allergen. A significant change did not occur in any of these indices of hypersensitivity.

In Figs. 1 and 2 also are shown unsuccessful attempts to maintain reduction of leucocyte sensitivity achieved through intravenous infusion of allergen by subcutaneous injection of large amounts of the allergen. The subject in Fig. 2 could be given 23,000 PNU subcutaneously the first day, beginning immediately after cessation of intravenous infusion with a dose of 10 PNU and increasing rapidly in eight injections that day until the eighth dose was 8000 PNU. There were no systemic reactions, presumably because of the reduction in leucocyte sensitivity. In other subjects in another study of intensive subcutaneous injections of allergen (Schumacher & May, 1972) we could not inject more than 300 PNU by gradually increased doses or reach a maximum dose of more than 100 PNU in the first day without inducing symptoms. In the subject in Fig. 2 10,000 PNU once a day was injected subcutaneously for another 11 days after intravenous infusion was stopped without checking the concordant return of leucocyte sensitivity to the original levels, and the histamine content of the leucocytes also rose to greater than the original value. Note that ANC of the serum was increasing, which may have been a factor in prevention of free allergen reaching a concentration in the circulation sufficient to release histamine from the basophils. Data from the subject in Fig. 1 indicate specific *alternaria* reaginic antibody in the serum may have increased slightly, and leucocyte sensitivity was temporarily reduced during intensive subcutaneous injection of allergen, but as ANC of the serum increased, the further subcutaneous injection of a large amount of allergen could not halt a concordant increase in leucocyte sensitivity. Note corresponding fluctuation of histamine content of blood leucocytes.

Data from two subjects in Table 3 and one subject in Fig. 3 deserve special consideration because the sensitivity of their leucocytes to histamine release by the allergens under study decreased, at some time, to the point of unresponsiveness to the highest concentration of allergen we customarily employed in the test. In the case of Subject 566 (Table 3) the leucocyte sensitivity decreased concordantly during infusion of allergen and recovered concordantly to the original sensitivity within 5 days after cessation of the infusion. Twenty days later the state of unresponsiveness appeared spontaneously or perhaps in conjunction with a respiratory infection. Unresponsiveness persisted for the remaining 56 days the subject was available for observation. Reaginic antibody titres in the serum were unchanged and bronchial sensitivity was slightly greater even after leucocytes had been unresponsive for 56 days.

With Subject 568 (Table 3) the first course of infusion of a small amount of allergen did not result in reduction of leucocyte sensitivity but a second course did. Three days after cessation of allergen infusion, leucocyte sensitivity was nearly back to the pre-infusion level. Twenty-one days later, blood was obtained for measurement of leucocyte histamine release prior to beginning another course of intravenous allergen. In this



Alt. (PNU)	110,000 i.v.	120,000 s.c. (10,000/day)
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ANC-Alt.	3	2	300	140
P.K. 1:10 (W/E) Alt.	7/25	10/32		15/40
P.K. undil. (W/E) Tim.	6/18	10/25		8/20
Skin test (W/E) Alt. 100 PNU/ml	8/25	7/20		

Subject 545

Fig. 2. Course of leucocyte sensitivity to histamine release by fixed concentrations of alternaria and timothy and level of histamine in blood cells are shown. In the first 3 days alternaria extract was administered intravenously, and as soon as this was discontinued, subcutaneous injections were begun and continued from Day 3 to Day 14. Amounts given are shown and details are in the text. Simultaneous measurements are shown of antigen neutralizing capacity of serum (ANC), reagin in serum (Prausnitz-Kustner procedure), and reaction to intradermal injection of the two allergens. Net diameters in mm of weal (W) and erythema (E) reactions in P.K. procedure and skin tests are shown. Results with 1/10 dilution of serum in P.K. tests and 100 and 1000 PNU/ml in skin tests were selected from observations with serial dilutions because the responses were representative and these dilutions were the highest that elicited definite reactions.

specimen leucocyte sensitivity was found to have decreased to a state of unresponsiveness. The intravenous injection of allergen was discontinued 30 hr after the start, during which period 1000 PNU alternaria extract was infused. The state of leucocyte unresponsiveness persisted at least 8 days, when the subject had to be discharged home. Again neither reagenic antibody titres in the serum nor bronchial sensitivity declined. In both subjects the histamine content of the leucocytes decreased with the decrease in leucocyte sensitivity during the periods of allergen infusion, but returned to the original levels and remained there throughout the subsequent period of leucocyte unresponsiveness.

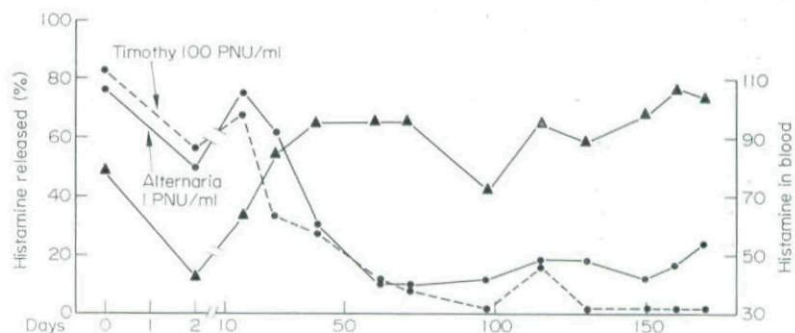
Similar phenomena occurred in Subject 444 (Fig. 3). The usual concordant decrease and recovery of leucocyte sensitivity in connection with infusion of allergen and

Table 3. Circumstances surrounding occurrence of leucocyte unresponsiveness*

Day		Histamine released from leucocyte (%)		Histamine in blood (ng/ml)	Antigen-neutralizing capacity	Prausnitz-Kustner procedure (serum diluted 1:10)		Bronchial provocation (PNU/ml for + response)
		Alternaria 0.3 PNU/ml	Ragweed 10 PNU/ml		Alternaria	Alternaria (mmW/mmE)	Ragweed (mmW/mmE)	Alternaria
Subject 566								
0	Moderate asthma							
1-3	Alternaria i.v. 89,500 PNU	77	88	34	2	12/40	8/20	4000
2		0	0	9				
		10 PNU/ml						
3		3	0	10				
		0.3 PNU/ml						
8		82	87	23				
28	Respiratory infection	10	0	62	50			
		1000 PNU/ml	1000 PNU/ml					
30		8	4	49				
36		9	11	44	35			
63		7	0	65				
86	Increased asthma	4	0	69		14/40	9/20	1000
Subject 568								
0	Moderate asthma							
1-3	Alternaria i.v. 2400 PNU	61	52	79	Alternaria	Alternaria	Ragweed	Alternaria
		0.3 PNU/ml	30 PNU/ml					
2		82	89	12				
3		72	72	29				
8		76	64	52				
9-11	2500 PNU							
10		65	49	41				
11	Respiratory infection	46	14	35				
14	Increased asthma	62	33	46				
35		6	1	50		12/37	7/25	
35-36	Alternaria i.v. 1000 PNU	1000 PNU/ml	1000 PNU/ml		22			
36		13	0	36				
43	Mild asthma	3	4	59				100

* Terms and abbreviations described in explanatory footnotes of Table 1 or in text.

its cessation are seen, along with corresponding fluctuation in histamine content of peripheral leucocytes. Then, perhaps in conjunction with a respiratory infection, the state of unresponsiveness gradually developed and persisted completely for timothy for over 100 days, while with alternaria (to which the leucocytes had been more sensitive) the sensitivity was markedly and persistently reduced but not to the state of unresponsiveness. ANC of serum increased for alternaria, the allergen infused, and not for timothy. One factor which may be involved is that the histamine content of the



i.v. R. Alt. 35,000 PNU

ANC Alt. 37 153 250
Tim. 1 1

P.K. 1:10 (W/E)	Alt.	10/42	10/47	12/47	11/46	12/40
	Tim.	7/25		8/20		7/25

Skin test PNU/ml	Alt. 100	6/25	6/20	5/18
	Tim. 1000	6/20	4/18	4/10

Bronch. P-Controls reactive

U.R.I.

Prednisone

Subject 444

Fig. 3. Course of leucocyte sensitivity to histamine release by fixed concentrations of alternaria and timothy and level of histamine in blood cells during and after intravenous infusion of alternaria. Expressions and amounts shown with respect to antigen neutralizing capacity of serum (ANC), P.K. procedure, and skin tests are explained in the legend to Fig. 2 or in the text.

leucocytes recovered after the decrease during infusion of allergen, and remained near the original level throughout the period of unresponsiveness. Neither skin tests nor reaginic antibody titres in serum decreased significantly during the fluctuations in sensitivity of peripheral leucocytes. Bronchial response to allergen could not be tested because hyperreactivity was so great, inhalation of diluent alone caused a significant reduction in expiratory flow throughout the period of observations.

Especially noteworthy is the finding that both the temporary reduction in leucocyte sensitivity during allergen infusion and the persistent state of unresponsiveness affected both allergens under study in a concordant manner. Also of interest is the finding that the unresponsive state was not associated with reduction of histamine content in the leucocytes as was regularly found during decrease of leucocyte sensitivity by allergen infusion.

The features of the three cases just described are the same as we observed in our original report of development of leucocyte unresponsiveness to two allergens during intensive subcutaneous injection of one (May & Aduna, 1971).

Table 4. Comparison of changes in leucocyte sensitivity with other indices of hypersensitivity

Leucocyte sensitivity (mean Δ)		Other indices (significant change/no. tested)				
		Skin test		P.K.		Bronchial prov.
Ag-1	Ag-2	Ag-1	Ag-2	Ag-1	Ag-2	Ag-1
-86%	-88%	0/6	0/5	0/6	0/5	0/7

In none of the other subjects in the present report given allergen intravenously were the leucocytes found to have become unresponsive to antigenic histamine release even though repeated studies of histamine release were done during periods of 4-15 weeks after the infusions.

Discussion

Whereas nonspecific desensitization of leucocytes may result from exposure *in vivo* to subthreshold concentration of an antigen to which they are sensitive (i.e. a concentration too low to release histamine), as suggested in our preliminary report (May *et al.*, 1972a) and by Lichtenstein & Levy (1972), the findings in this more extensive study indicate a major mechanism is actual degranulation of basophils and histamine release. This would lead to elimination of the population of basophils sensitized and in readiness to react with the antigen. The leucocytes of the subjects studied varied in degree of sensitivity, and both small and large amounts of allergen were infused at different rates. Nevertheless, in all cases concordant decrease in leucocyte sensitivity was accompanied by histamine release, as evidenced by concomitant decrease in basophil count and histamine content of leucocytes, increased urinary excretion of histamine, and clinical manifestations of mediator release (wheezing and urticaria). Upon cessation of allergen infusion, leucocyte sensitivity and content of histamine returned in unison to preinfusion levels. In three relatively insensitive subjects in whom these features did not appear, leucocyte sensitivity was not decreased by infusion of allergen in amounts and at rates which probably achieved only subthreshold concentration of allergen in the circulation.

In those instances where persistent, complete loss of leucocyte sensitivity or unresponsiveness developed apart from injection of allergen, histamine content of the leucocytes was not decreased. The decline to unresponsiveness was gradual, requiring days to weeks (Fig. 3). The mechanism of this type of reduction in leucocyte sensitivity is obscure, but in some cases preceding injection may play a role. In earlier papers (Schumacher & May, 1972; May *et al.*, 1972b) we have considered the true incidence of development of leucocyte unresponsiveness and the necessity of using an optimal system in determining histamine release or lack thereof. In our previous experience complete or persistent unresponsiveness developed infrequently, during or without injection therapy. Furthermore, although the state of unresponsiveness may persist for months, it is not necessarily permanent, and, if it develops in association with a course of injection therapy, may not be reproduced by another course of injections after sensitivity of leucocytes has recovered (May & Aduna, 1971; May *et al.*, 1972b).

In both the brief reduction of leucocyte sensitivity induced by injection of allergen and the persistent form of unresponsiveness just mentioned, the reduction and the recovery in sensitivity to unrelated antigens occurred concordantly. Also we found that for both types a dissociation existed between the loss of leucocyte sensitivity and lack of evidence of loss of hypersensitivity by other indices, such as skin test, bronchial provocation, and reaginic antibody titre in serum.

In the more persistent form of unresponsiveness of leucocytes which develops apart from injection of allergen (Table 3, Fig. 3) the histamine content of leucocytes is not decreased but the loss of sensitivity is gradual, so the number of circulating basophils may be maintained even though sensitized ones are being steadily degranulated. The long duration makes it unlikely that a biochemical intermediate involved in histamine release is exhausted, because a generation of basophils remains in the circulation only a matter of hours and would be replaced by new cells. (The rapid recovery of leucocyte sensitivity to the pre-infusion level after cessation of infusion of allergen is in keeping with this.) The titres of specific reaginic antibodies in the serum are undiminished, and are able to passively sensitize cells of normal persons (May & Aduna, 1971). We do not know whether in the unresponsive state the basophils have lost capacity to fix reaginic antibody, whether some other moiety has occupied receptor sites for reaginic antibody, or if something may be interfering with combination between cell-bound antibody and antigen or release of histamine.

Ultimately, it may be found that the types of concordant fluctuation in leucocyte sensitivity described do not differ in fundamental mechanism but depend only on the rate at which basophils may be degranulated or restored in various circumstances, such as: (1) during intravenous infusion of allergen versus more gradual exposure naturally or to allergen injected subcutaneously; (2) large or small dosages; or (3) in early versus late phases of injection therapy (as serum content of specific antigen-neutralizing antibodies increases).

A most intriguing aspect of concordant fluctuation in basophil sensitivity to histamine release by unrelated antigens is the possibility that the same population of basophils in the circulation serves to express hypersensitivity to perhaps all antigens to which a person is sensitive. This would mean that only basophils of this population are fully prepared by being equipped biochemically for the process of histamine release and bearing an adequate number or arrangement of specific reaginic antibodies for all antigens involved in the person's immediate hypersensitivity reactions. What fraction of the total population of basophils are prepared to react may well be variable, but in the cases of greatest reduction in sensitivity by intravenous infusion of allergen, a considerable portion of histamine-containing cells (presumably basophils) apparently remain intact (Table 1, Column 5). Another possibility is that some factor is released by the injected allergen which inhibits histamine release by unrelated allergens. Against this is the consideration that washed leucocytes are used in the *in-vitro* method for measurement of histamine release.

Lichtenstein and Levy have also reported concordant fluctuation in sensitivity of peripheral leucocytes in subjects receiving subcutaneous injection of allergen in a paper bearing the delusive title: 'Is "desensitization" for ragweed hayfever immunologically specific?' (Lichtenstein & Levy, 1972). The implication they drew from concordant decrease in sensitivity of peripheral leucocytes to unrelated allergens during injection of one allergen was that clinical reactions to unrelated allergens may also be found to decrease concordantly and nonspecifically after injection of one allergen.

This implication reflects the oft-stated opinion of these investigators that peripheral leucocyte sensitivity and clinical symptoms from an allergen are closely correlated. While there may be a significant statistical correlation, especially at high levels of sensitivity, the gross exceptions may be clearly seen in their data (Lichtenstein *et al.*, 1966). In a recent paper by Lichtenstein, Norman & Winkenwerder (1971), examples are presented of lack of reduction in clinical symptoms even when leucocytes became completely insensitive or unresponsive to histamine release by allergen.

There is no doubt about the specificity of antigenic release of histamine from leucocytes of hypersensitive persons (May & Aduna, 1971; May *et al.*, 1970a, b, 1972a; Schumacher & May, 1972). However, if the population of basophils having the capacity to release histamine with one antigen also bears the capacity to release histamine with all other antigens to which hypersensitivity exists, and this capacity is exclusively restricted to this population, release of histamine or degranulation by any one antigen to which the population of basophils is sensitive, would proportionately reduce the basophils capable of reacting with any antigen participating in immediate hypersensitivity reactions in a particular subject.

Temporary anergy in humans or animals immediately after anaphylactic reactions is probably due to degranulation of all sensitized basophils, mast cells, or other cells involved, and/or to neutralization of the supply of specific antibody, leaving them unable to react rather than nonspecifically desensitized in a true immunologic sense. The supply of specific antibody and sensitized cells in readiness to respond only to the specific antigen is usually soon replaced—the underlying specific hypersensitivity has persisted (Fellner *et al.*, 1970).

Analogous development of nonspecific unresponsiveness occurs locally when antigen is injected repeatedly into the same site in the skin of a sensitized subject (Cooke, 1922; Bowman & Walzer, 1953). Here altered vascular reactivity may also play a role, as repeated injections of histamine in the same site caused unresponsiveness to develop to histamine and also to antigen.

The meaning of the word 'desensitization' is clear and simple but the connotations can be confusing. Desensitization would be most properly applied to prolonged elimination of the state of hypersensitivity from an individual, either specifically or nonspecifically. Desensitization in this sense would undoubtedly lead to relief of clinical allergic manifestations, specifically or generally as the case may be. Misleading connotations are possible when desensitization is used to describe interference with only a portion of the allergic process, such as degranulation of basophils, that may not affect all the tissues and so may not prevent the appearance of symptoms. When an essential element in the allergic reaction is eliminated from some cells by the specific action of any antigen, the action of other antigens on that process will seem to be prevented nonspecifically, even though specific antigen-antibody reactions may still take place with other tissues.

Our observations indicate peripheral leucocytes are vulnerable to factors causing fluctuations in leucocyte sensitivity unaccompanied by similar effects on the general state of hypersensitivity. Therefore the usefulness of peripheral leucocytes for monitoring injection therapy and other manipulations aimed at altering the hypersensitive state seems subject to serious limitation. Perhaps the most one can say is that unless the peripheral leucocytes are 'desensitized' by injection therapy, it is unlikely any of the tissues will be. Furthermore, reduction or elimination of peripheral leucocyte sensitivity cannot be regarded as a sufficient goal for injection therapy. The dissociation

described also suggests differences in behaviour of basophils in the circulation and mast cells in the tissues.

Clinical allergists will probably be struck by the demonstration that so much allergen can be infused intravenously within 3 days without mishap. Actually the allergen was infused intravenously by our procedure under better control than is possible with subcutaneous injections, from which allergen may reach the circulation erratically in uncontrollable amounts and rates. Clinical allergists should also note that the large amounts of allergen injected caused only an evanescent reduction in sensitivity of peripheral leucocytes and no significant effect on bronchial or cutaneous sensitivity or reaginic antibody titres in serum. We wish to emphasize that experimental intravenous infusion of allergen should not be regarded as a suitable form of therapy at this time.

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