

Significance of concordant fluctuation and loss of leukocyte sensitivity to two allergens during injection therapy with one nonspecific desensitization

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Concordant, nearly parallel fluctuations in leukocyte sensitivity to 2 unrelated allergens were repeatedly observed during injection therapy with one of the allergens. In one case, complete loss of sensitivity or unresponsiveness to 2 unrelated allergens occurred during injection therapy with one. This nonspecific desensitization is considered to result from exposure of the sensitized leukocytes to concentrations of one of the allergens below the concentration required for detectable histamine release. This explanation is supported by data obtained by exposure of leukocytes in vitro to subthreshold concentrations of allergen, which resulted in decreased response in histamine release by the allergen used for desensitization and an unrelated allergen. In addition, intravenous infusion of allergen in low concentration into children likewise induced nonspecific desensitization of circulating leukocytes to 2 unrelated allergens. Concordant fluctuations and occasional nonspecific loss of leukocyte sensitivity to unrelated allergens during customary subcutaneous injection therapy may result from uneven or inadequate absorption of free allergen from the subcutaneous tissues into the circulation, thereby reaching desensitizing concentrations intermittently. This phenomenon calls for caution in assuming that the fluctuations of peripheral leukocyte sensitivity during injection therapy are necessarily a reflection of sensitivity of cells in the tissues.

In the course of studies of injection therapy with an allergen extract, we have encountered remarkably concordant fluctuations in leukocyte sensitivities to the allergen injected and an unrelated allergen when the 2 were measured simultaneously. Recently we¹ reported an example of simultaneous loss and subsequent recovery of leukocyte sensitivity to 2 unrelated allergens during intensive injection therapy with one. Sporadically, in the previous 5 years, we had measured leukocyte sensitivity to 2 allergens simultaneously at frequent intervals and in 6 subjects had observed concordant increases and

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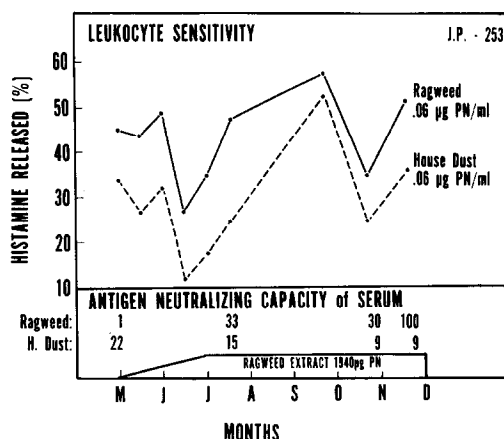


FIG. 1. Concordant fluctuation of leukocyte sensitivity (percentage histamine release by fixed concentration of allergen) to ragweed and house dust extracts in subject receiving subcutaneous injections of ragweed extract. Antigen neutralizing capacity of serum (ANC) for ragweed increased during the injection of ragweed extract, while decreasing for house dust. Units used to express the magnitude of ANC are described in the paper on the method of determination.⁵

decreases in sensitivity during periods of many months. An example from these unpublished data is shown in Fig. 1. Stimulated by these observations, a systematic determination of fluctuations in leukocyte sensitivity to 2 unrelated allergens was undertaken in conjunction with a comprehensive study of relatively intensive injection therapy. As will be shown, a striking concordance in fluctuations of leukocyte sensitivity to the 2 allergens was also observed in that study, other aspects of which will be reported separately.²

The present report is concerned principally with the significance of concordance in fluctuations of leukocyte sensitivity to unrelated allergens, especially with what appears to be a nonspecific effect of injection of allergen extract on leukocyte sensitivity to antigenic histamine release. Also, consideration is given to the bearing of the findings on nearly complete loss of leukocyte sensitivity which sometimes develops during injection therapy, as well as spontaneously.^{1, 8, 9}

The hypothesis developed to guide investigation of this phenomenon is that when leukocytes (basophils) sensitized with reaginic antibodies to multiple antigens are exposed to a concentration of one of the antigens below the concentration required to cause detectable histamine release (subthreshold), subsequently the leukocytes may be found to exhibit a concordant decrease in sensitivity to histamine release when tested with appropriate concentrations of any of the antigens to which they had originally been sensitive, as if a generalized nonspecific desensitization had occurred.

The data presented are derived from 2 approaches: (1) *in vitro* desensitization of leukocytes, based in part on an isolated comment by Liechtenstein⁸ that nonspecific desensitization could be induced by incubation of leukocytes with a subthreshold concentration of an antigen to which they were sensitive;

(2) preliminary trials of in-vivo induction of nonspecific decrease in leukocyte sensitivity by intravenous injection of an allergen to which the individual was sensitive. The intravenous route of injection was necessary for dependable achievement of a subthreshold concentration of allergen in the circulation.

METHODS AND MATERIALS

Antigenic release of histamine from leukocytes and antigen-neutralizing capacity of serum were determined by methods already described.^{4, 5} Fluctuation of leukocyte sensitivity is followed by noting the percentage of histamine released by a fixed concentration of antigen in serial observations. Terms and units are explained in the footnotes of the tables and in the legends of the figures.

Allergen extracts used in laboratory procedures were freeze-dried products produced commercially by Greer Laboratories, Lenoir, N. C., or Center Laboratories, Port Washington, N. Y. These were reconstituted in preservative-free buffer so as to maintain uniform potency as described in the papers on methods.^{4, 5} Allergen extracts used in subcutaneous and intravenous treatment were stock solutions in buffer with phenol as usually provided for injection treatment by the Greer Laboratories, with care being taken to maintain potency as previously described. Dilutions for subcutaneous injection were made with buffer containing phenol and for intravenous injection with 0.9 per cent sodium chloride solution.

Leukocytes from peripheral blood of allergic adults and children were used in in vitro studies.

Subjects

Current studies of persons receiving injection therapy with allergen extracts were with children in residence for long-term care in the National Jewish Hospital and Research Center. Preliminary trials of intravenous injection of allergen extracts were carried out with consent of fully informed parents and under constant immediate supervision of the investigators in the Children's Clinical Research Center in the Department of Pediatrics, University of Colorado Medical Center.* The protocols for the intravenous injection of allergen were approved by the Committees on Human Experimentation of the National Jewish Hospital and Research Center and the University of Colorado Medical Center.

Procedures

In vitro desensitization of leukocytes. Two unrelated allergens were selected for study from preliminary determination of concentration-response curves for allergenic release of histamine from leukocytes with the published methods. The allergens selected generally gave at least 50 per cent release of leukocyte histamine. From the concentration-response curves, the concentrations below the level required for detectable histamine release (subthreshold) and the concentrations required to give 40 to 80 per cent release could be selected for use in the ensuing procedure.

Desensitization was attempted by incubation of sensitive leukocytes with subthreshold concentrations of one allergen. Then the sensitivity of these leukocytes and of untreated aliquots of the leukocytes was determined toward the same or one or more unrelated allergens added at concentrations previously found to cause abundant histamine release. At first we removed the desensitizing allergen at the end of incubation by centrifuging the leukocytes, discarding the supernate, and resuspending the leukocytes in buffer before the addition of the test allergens. This was found by experiment to have no effect on the results, and our final procedure called for addition of suitable concentrations of test allergens directly to the leukocyte suspension at the end of the desensitizing incubation. Normal serum was not included in the

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mixture in the desensitizing step, but 8 per cent group AB normal human serum was introduced before addition of test concentrations of the allergens to provide optimal conditions for histamine release. The following typical protocol permitted trial of 2 subthreshold concentrations of allergen in the desensitization step, and subsequent testing of 2 concentrations of 2 allergens with leukocytes from 18 ml. blood.

Protocol. Eighteen milliliters of blood was collected in a plastic syringe containing about 0.2 ml. heparin (1,000 U. per milliliter). To facilitate separation of the leukocytes, 6 ml. heparinized blood was layered over 3 ml. methyl cellulose-sodium diatrizate mixture⁶ previously placed in each of three 17 × 100 mm. capped polystyrene tubes (Falcon No. 2001). As soon as sharp separation of the plasma leukocytes occurred, the layers from the 3 tubes were aspirated, pooled, and then centrifuged at 180 G for 10 minutes. The plasma was decanted thoroughly. The cells were resuspended in 12.5 ml. Tris-Ca-Mg buffer⁴ for use as follows in Protocol I:

PROTOCOL I. Desensitization with subthreshold concentrations of allergen

Tube No.	4	4A	5	5A	6	7	8	9	10	11	12	13
Leukocyte suspension	1.0 ml.											
Allergen—Conc. 1					0.1	0.1			0.1			
Allergen—Conc. 2							0.1	0.1		0.1		
Tris-CM buffer	0.1	0.1	0.1	0.1							0.1	0.1

Incubate 20 minutes at 37° C. Centrifuge tubes 5, 10, 11 at 700 G for 10 minutes.

Histamine analysis on 0.9 ml. of supernates and on 0.9 ml. cell suspension in tube 4 for total histamine content, using published method.⁴ These analyses detect any release during the desensitization step.

To remainder of tubes, add as follows in Protocol II:

PROTOCOL II. Testing with allergens in concentrations previously determined to give about 40 to 80 per cent histamine release with untreated leukocytes

Tube No.	4A	5A	6	7	8	9	12	13
Normal AB serum		0.1 ml.						
5% Normal serum	0.2							
Tris CM buffer	0.1							
Allergen—Conc. 1			0.1		0.1		0.1	
Allergen—Conc. 2				0.1		0.1		0.1

Incubate 40 minutes at 37° C. Centrifuge tubes 5A to 13 at 700 G for 10 minutes.

Histamine analysis on 1.0 ml. of supernates and 1.0 ml. cell suspension in tube 4A for total histamine content.⁴ Tubes 12 and 13 give the release from untreated leukocytes.

Internal and external histamine standards and blanks for fluorescence in serum or allergens are also run as described in previous papers^{4, 5} in which calculations are also discussed.

In vivo desensitization of leukocytes. An allergen extract concentration of 0.01 μ g protein nitrogen (PN) per milliliter (1 PNU per milliliter) was used at the start, and, as increases in rate of administration were tolerated, concentrations of up to 1 μ g PN per milliliter (100 PNU per milliliter) were employed. The solutions were administered through ordinary equipment for constant intravenous infusion. Rather than depending on gravity to control the rate, mechanical pumps were employed (Harvard Instrument Co., Millis, Mass., Model 2681) which could be adjusted to deliver from 1.8 to 10 ml. per hour with dependable precision. Thus, rates of administration of allergen began at 0.018 μ g PN (1.8 PNU) per hour and progressed to as much as 10 μ g PN (1,000 PNU) per hour as tolerated.

The children given allergen intravenously included highly sensitive persons subject to frequent severe attacks of asthma. They were having moderate symptoms even though receiving large dosages of theophylline (up to 10 mg. per kilogram body weight every 6 hours) and in some cases corticosteroid as well (up to 40 mg. prednisone every other day). Medications were continued during the study, but in the past this amount of medication had not prevented severe asthma from recurring occasionally. Repeated preliminary determinations had shown that these dosage regimens of the medications did not affect in vitro allergenic histamine release from leukocytes.

After collection of preinfusion data, the intravenous infusion of allergen extract was

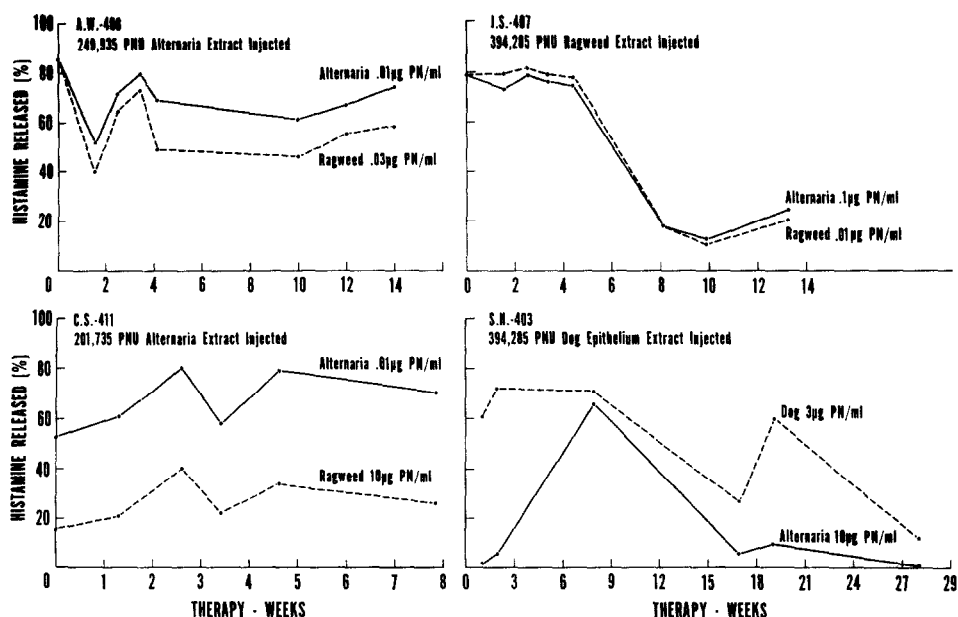


FIG. 2. Concordant fluctuation of leukocyte sensitivity to 2 allergen extracts in subjects receiving intensive injection of extract of one of the allergens subcutaneously.

cautiously started. Beginning at about $0.018 \mu\text{g}$ PN per hour, the rate was doubled at 30 to 60 minute intervals until reactions occurred. These were usually urticaria or wheezing. The rate of infusion was then kept constant or reduced slightly while 0.2 ml. of $1:1,000$ epinephrine solution was given subcutaneously. One dose usually relieved symptoms within 20 minutes, and subsequently increases in rate of infusion could proceed. Sometimes, in addition, 25 mg. diphenhydramine was given orally for a sustained effect. The aim was to achieve a subthreshold concentration of allergen in the circulation. Therefore, the rate of administration was not increased further when mild symptoms of histamine release tended to persist or reappear with each increase. These symptoms were taken as an indication that the subthreshold concentration was being exceeded. The intravenous infusions were given continuously for 96 hours, and the amount of allergen administered each 24 hours is shown in the figures and tables. As mentioned, up to $10 \mu\text{g}$ PN ($1,000 \text{ PNU}$) per hour was given.

No serious or anaphylactic reactions occurred. Examination of blood cells and urine revealed no evidence of renal or other disturbance. Nevertheless, utmost caution and constant supervision should be exercised by experienced clinicians in such intravenous administration of allergen to highly sensitive asthmatic children.

RESULTS

Confirmation of concordance in fluctuations of leukocyte sensitivities to unrelated allergens

Fig. 2 presents the results of simultaneous determinations of leukocyte sensitivity to histamine release by 2 unrelated allergens obtained during a study of intensive subcutaneous injection therapy with one of the allergens. The serial values for leukocyte sensitivity are shown in 4 subjects from whom complete data for 2 unrelated allergens could be obtained. The allergens injected subcutaneously and the total doses administered during the periods of observation are indicated on the charts. Additional details of the injection therapy

TABLE I. Decrease in leukocyte sensitivity to antigenic release of histamine induced by subthreshold concentration of antigen

Experi- ment No.	Desensitization			Testing			Control histamine release† (%)	Change§ (%)
	Antigen	Concen- tration* (μ g PN/ ml.)	Hista- mine release (%)	Antigen	Concen- tration† (μ g PN/ ml.)	Hista- mine release (%)		
1-395J	Alternaria	3×10^{-5}	0	Alternaria	3×10^{-2}	27	66	-59
2-381G	Ragweed	1×10^{-4}	1	Ragweed	1×10^{-2}	53	82	-35
3-381F	Timothy	1×10^{-3}	0	Timothy	1×10^{-1}	16	60	-73
4-403C	Dog dander	1×10^{-3}	0	Dog dander	1×10^{-1}	21	53	-79
5-405D	Timothy	1×10^{-3}	0	Timothy	1×10^0	19	46	-58
6-405D	Timothy	1×10^{-3}	0	Alternaria	1×10^{-1}	67	80	-16
7-441I	Alternaria	1×10^{-3}	5	Alternaria	1×10^{-2}	14	50	-72
8-441I	Alternaria	1×10^{-3}	5	Ragweed	1×10^{-1}	33	59	-45
9-405H	Alternaria	1×10^{-5}	3	Alternaria	1×10^{-2}	22	73	-70
10-405G	Alternaria	1×10^{-5}	3	Timothy	1×10^{-2}	9	32	-72
11-405H	Alternaria	1×10^{-5}	3	Anti-IgE	1:10,000	18	36	-50

*Concentration antigen used for desensitization (μ g PN = micrograms protein nitrogen).

†Concentration antigen used after desensitization.

‡Histamine released from untreated leukocytes by concentration of antigen used in testing.

§Per cent decrease in histamine release by antigen after desensitization compared to control.

||1:10,000 dilution of commercial anti-IgE antiserum.

TABLE II. Relations between desensitizing and testing concentrations of antigen (Expt. 403C.; antigen = dog dander)

Controls (untreated leukocytes)				Desensitization concentrations			
Antigen (μ g PN/ml.)	0.03	0.1	0.1	μ g PN/ml.	0.0001	0.001	0.01
Histamine release (%)	21	53	85	Release	0	0	0
				Desensitization† (μ g PN/ml.)	0.0001	0.0001	0.0001
				Test‡ (μ g PN/ml.)	0.03	0.1	1
				Release (%)	0	19	66
				Change§ (%)	-100	-64	-22
				Desensitization (μ g PN/ml.)	0.001	0.001	0.001
				Test (μ g PN/ml.)	0.03	0.1	1
				Release (%)	0	11	44
				Change	-100	-79	-48

*Per cent of total histamine released by concentration dog dander shown.

†Concentration antigen used for desensitization.

‡Concentration antigen used after desensitization.

§Per cent decrease in histamine release by antigen after desensitization compared to controls.

and other information concerning the study of these subjects may be found in a separate paper.² The nearly parallel concordance in fluctuations in leukocyte sensitivities to the injected and unrelated allergens is evident in Fig. 2.

In vitro desensitization of leukocytes

Data in Table I show that leukocyte sensitivity was reduced 35 to 80 per cent by treatment of the leukocytes with subthreshold concentrations of allergen. Experiments 5 through 11 show that the desensitization affects sensitivity to allergens unrelated to the allergen used for desensitization, although seemingly to a lesser degree. The desensitizing concentrations were generally $\frac{1}{10}$ to

TABLE III. Effect of relative concentrations of antigen used for desensitization and testing

Experiment No.	Desensitization			Testing			Control histamine release (%)	Change (%)
	Antigen	Concentration (μ g PN/ml.)	Histamine release (%)	Antigen	Concentration (μ g PN/ml.)	Histamine release (%)		
1-444F	Alternaria	0.0001	0	Alternaria	0.01	29	30	0
2-444F	Alternaria	0.0001	0	Alternaria	0.1	74	76	0
3-444F	Alternaria	0.001	0	Alternaria	0.01	18	30	-40
4-444F	Alternaria	0.001	0	Alternaria	0.1	56	76	-26

Headings of columns defined in Table I.

$\frac{1}{1,000}$ of the test concentrations which gave 50 to 80 per cent histamine release with control or untreated leukocytes.

The degree of desensitization is apparently influenced by relations between the desensitizing and testing concentrations of allergen. In the experiment shown in Table II desensitization was 22 to 100 per cent depending on which combination of concentrations of allergen was used in desensitization and testing. The same can be seen in the experiment in Table III in which either no desensitization or up to 40 per cent occurred depending on the combination of concentrations of allergen used. The data in Tables II and III also suggest that the desensitizing effect of a given concentration can sometimes be partly overcome if a large enough concentration is used in the testing phase. Compare results with increasing test concentrations of allergen in Table II and note items 3 and 4 in Table III.

In vivo desensitization of leukocytes

The results of intravenous infusion of allergen extract are present in Figs. 3 and 4. Changes in leukocyte sensitivity to 2 allergens are shown. Typical concentration-response curves from experiments with one subject are given in Fig. 3, and by determination of such curves in all subjects the percentage histamine released by a fixed concentration of allergen (leukocyte sensitivity) was followed in serial observations. In Fig. 4 changes are shown in leukocyte sensitivity to 2 allergen extracts for the 3 subjects infused intravenously with one of the allergen extracts as indicated. Two charts for Subject T. D. -444 show the findings from successive periods I and II for separate infusions of 2 unrelated allergens in the same subject. In Fig. 4 it is easily seen that in all cases intravenous infusion of one allergen extract caused decrease in leukocyte sensitivity to that allergen and concordantly to the unrelated allergen being followed simultaneously. Usually leukocyte sensitivity was decreased about 50 per cent in the 3 day period of infusion of the amounts of allergen shown in the charts in Fig. 4. Leukocytes of Subject K. B. -515 became close to unresponsive. After cessation of the infusion, sensitivity of leukocytes to both allergens was recovered, also concordantly, within a few hours to a few days. The fluctuations in sensitivity to 2 allergens induced by intravenous infusion of one allergen were of similar magnitude and as nearly parallel as had been observed during intensive subcutaneous injection therapy (Fig. 2).

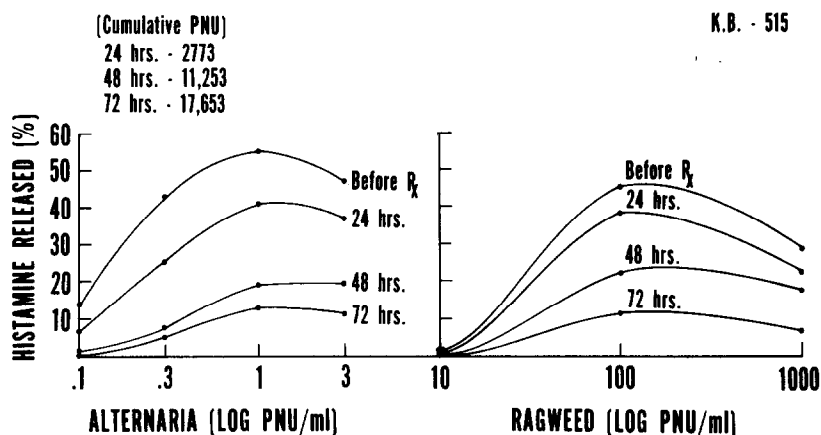


FIG. 3. Changes in leukocyte sensitivity to histamine release by *Alternaria* and ragweed extracts during intravenous administration of *Alternaria* extract. Concentration-response curves before intravenous therapy and at 24 hour intervals during infusion are shown, along with the cumulative amount infused.

DISCUSSION

The induction of nonspecific desensitization by exposure of leukocytes in vitro to subthreshold concentrations of allergen confirms and extends an observation by Lichtenstein.⁸ Intravenous infusion of allergen into hypersensitive children at a rate which caused no more than occasional mild symptoms of histamine release may be considered to have achieved subthreshold concentrations of allergen in the circulation. This in vivo exposure of leukocytes to a subthreshold concentration of allergen likewise induced nonspecific desensitization.

The resemblance of the concordance and nonspecificity in intravenously induced changes in leukocyte sensitivity to the nearly parallel fluctuations in leukocyte sensitivity we have observed during subcutaneous injection therapy with allergen extracts is highly suggestive of identical basic phenomena. The findings support our hypothesis that such concordant fluctuations or loss of leukocyte sensitivity to 2 unrelated allergens during injection therapy with one allergen may be due to nonspecific desensitization through exposure of the leukocytes to a subthreshold concentration of an allergen to which they are sensitive.

To account for the sporadic increases and decreases in leukocyte sensitivity we observed during customary subcutaneous injection therapy and the infrequent and temporary occurrence of loss of leukocyte sensitivity or unresponsiveness, irregular entrance of allergen into the circulation from the subcutaneous space may be assumed. Not only is the rate of absorption of allergen from the subcutaneous injections uncertain and probably slow (or systemic reactions would be more frequent), but also dosages administered are quite variable. What concentration of free allergen may be reached in the circulation will also be affected by the titer and avidity of specific antibodies in serum competing with cell-bound antibody for combination with allergen absorbed from sub-

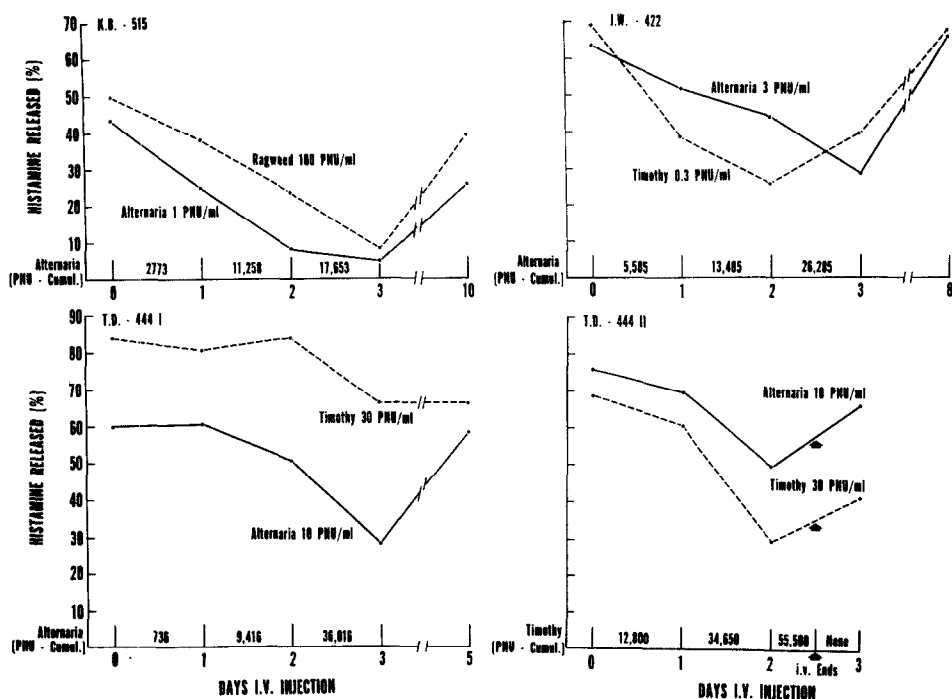


FIG. 4. Concordant fluctuations in leukocyte sensitivity to 2 unrelated allergen extracts during intravenous infusion of one of the allergen extracts. Which allergen was being infused intravenously and the cumulative amount are shown.

cutaneous injections. These considerations raise the possibility that so-called "blocking" antibody in the serum may actually act to prevent leukocyte desensitization by competing with cell-bound antibody for combination with allergen reaching the circulation. At least we⁷ and others⁸ have found there is no relation between a high level of antigen-neutralizing capacity of serum and leukocyte desensitization (Fig. 1).

Injection of allergen directly into the vein obviates erratic absorption from subcutaneous injections, but the dynamic interplay between allergen and antibodies in the serum or bound to cells may still influence the concentration of free allergen to which the leukocytes are exposed, thus the degree of desensitization which may occur.

Our finding that desensitization may depend in part on a relationship between desensitizing and testing concentrations of allergen (Tables II and III) would add to the complexity of conditions required for appearance of desensitization or loss of leukocyte responsiveness during customary subcutaneous injection therapy.

We found in our preliminary experience that reduction of leukocyte sensitivity induced by brief intravenous injection of allergen was quite temporary and the original sensitivity might be restored within hours after cessation of continuous intravenous injection of allergen. This may be one reason that wide fluctuations and infrequent and temporary loss of leukocyte sensitivity have

been observed during ordinary subcutaneous injection therapy. Proper conditions for regular and lasting induction of nonspecific desensitization of leukocytes, to a marked or unresponsive degree, are still to be identified.

The mechanism of this means of induction of nonspecific desensitization of peripheral leukocytes will probably have to be understood before optimal conditions for lasting effects are likely to be determined and potential for clinical usefulness will be discernible. Because the nonspecific fluctuations in peripheral leukocyte sensitivity described may not necessarily be accompanied by corresponding changes in sensitivity of other cells in tissues, such as mast cells, caution should be exercised in using reduction in peripheral leukocyte sensitivity alone as an index of clinically significant response to injection therapy. Lack of correlation between leukocyte sensitivity and skin sensitivity or clinical symptoms was found in some of our studies^{2, 7} and by others.^{8, 9}

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