Dose response relationships and interference of simultaneous skin tests in delayed hypersensitivity

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SYNOPSIS The relationship between the intensity of the delayed hypersensitivity reaction and the dose of antigen used for its elicitation change with time after sensitization. At three weeks the reactions to 100 μ g of egg albumen were little different from those to 1 μ g but at 14 weeks 100 μ g gave much stronger reactions. This means that to follow the development of a delayed hypersensitivity response several antigen doses should be used.

If two skin tests were performed simultaneously in the same individual, the stronger one suppressed the weaker one, regardless of whether two different doses of the same antigen or two different antigens were used. Consequently, not more than one skin test at a time should be performed.

Delayed hypersensitivity is detected by intradermal antigen injection in both clinical and experimental immunology. Skin reactivity can be quantitatively approached either by evaluation of the erythema and induration provoked by a given dose of antigen or by determination of the smallest amount of antigen eliciting a positive reaction.

The characteristic mononuclear cell infiltrate of delayed hypersensitivity is hematogenous and contains a minority of specifically sensitized cells (Kosunen, Waksman, Flax, and Tihen, 1963; McCluskey, Benacerraf, and McCluskey, 1963). Studies of correlates *in vitro* of delayed hypersensitivity have shown that the underlying sequence of events can be divided in a specific and non-specific part. At first antigen is recognized by specifically sensitized lymphocytes, and later non-specific lymphocytes; soluble lymphocyte products and macrophages complete the visible reactions (Pick and Turk, 1972; Ruddle, 1972).

In the hope of more results, several skin tests are often performed in one individual at the same time. The aim of this study was to confirm our experience that simultaneous skin reactions interfere and, therefore, do not reveal the true reactivity.

Materials and Methods

ANIMALS AND SENSITIZATION

White male guinea pigs weighing 400 to 500 g

were used. A saline solution of hen egg albumen (Sigma, St Louis, Missouri) was mixed (1:1) with complete Freund's adjuvant (CFA) consisting of 1.5 ml of Arlacel A (Mannide Monooleate, Atlas Powder Co, Wilmington, Delaware), 8.5 ml of Bayol F (Esso), and 60 mg of heat-killed tubercle bacilli. The animals were injected intradermally in the neck with 0.1 ml of the adjuvant emulsion containing 5 μ g of egg albumen.

SKIN TESTING

The guinea pigs were injected in the preshaven flank with 0.1 ml volumes of saline solutions of egg albumen or tuberculin-purified protein derivative (PPD) (Purified Tuberculin, Statens Seruminstitut, Copenhagen). The skin reactions were recorded 24 hours later. The erythema was measured with a ruler, and the induration was evaluated from - to + + + + by palpation between two fingers and, in part of the experiment, it was measured with a caliper as the increase in skin double thickness (recorded immediately before the intradermal injection and 24 hours afterwards).

STATISTICAL TREATMENT

The arithmetic means of numerical values were compared in Student's *t* test. The median was chosen as the central value for the degrees of induration, which were compared in the nonparametric rank-sum test (Dixon and Massey, 1957). The compared groups were tested at the same time.

Results

DOSE RESPONSE RELATIONSHIP IN DELAYED SKIN REACTIVITY

Groups of guinea pigs were sensitized with 5 μ g of egg albumen in complete Freund's adjuvant. Three weeks later they were injected intradermally with 100 μ g, 10 μ g, 1 μ g, 100 ng, or 10 ng of egg albumen. A positive dose response relationship was found, but a tenfold (unpublished) or hundredfold change in the antigen dose caused surprisingly little change in both the erythema and induration at 24 hours (table I). Fourteen weeks after sensitization the reactivity was more clearly related to the dose of egg albumen (table II).

INTERFERENCE OF SIMULTANEOUS DELAYED REACTIONS TO TWO DOSES

Guinea pigs were skin tested with various doses of egg albumen simultaneously with 100 μ g. The strong 24-hour reaction to 100 μ g of egg albumen suppressed significantly the reactions to lower doses of the same antigen, as compared with similar tests performed alone (table I). A 1000-fold dose of egg albumen was needed to detect positive reactions under the influence of the simultaneously injected 100 μ g. Two tests with 100 μ g did not suppress each other significantly (table I).

INTERFERENCE OF DELAYED REACTIONS TO DIFFERENT ANTIGENS

Fourteen weeks after sensitization with 5 μ g of egg albumen in complete Freund's adjuvant, guinea pigs were skin tested with 5 μ g of egg albumen or 10 μ g of PPD either alone or simultaneously with 100 μ g of egg albumen. Table II shows that the strong delayed reaction suppressed significantly the weaker ones to both egg albumen and PPD to roughly the same extent.

Discussion

The present data show that (a) the dose response relationship in the elicitation of delayed skin reactivity changes with time after sensitization; (b) stronger delayed reactions suppress simultaneously developing weaker ones; and (c) the suppression is not antigen specific. The practical significance of these findings is clear: not more than one skin test should be performed at a time.

Three weeks after sensitization, raising the intradermal dose of egg albumen from 1 to 100 μ g had little effect on the intensity of the subsequent delayed reaction. At 14 weeks, 100 μ g gave markedly stronger reactions than 5 μ g. This suggests that there is a limiting factor (other than the antigen)

Skin Test		Delayed Skin Reactivity		Value of P
Dose of Egg Albumen		Test Alone	Test Simultaneously with 100 μ g of Egg Albumen	
100 μ g	Er	25 $\pm$ 1	23 $\pm$ 2	>0.05
	Ind	++	++	>0.05
	Th	2.14 $\pm$ 0.18	1.67 $\pm$ 0.21	>0.05
	No.	19	6	
10 μ g	Er	ND	17 $\pm$ 2	
	Ind	ND	+	
	Th	ND	0.80 $\pm$ 0.33	
	No.	ND	10	
1 μ g	Er	22 $\pm$ 1	9 $\pm$ 1	<0.001
	Ind	++ to +++	—	<0.001
	Th	1.96 $\pm$ 0.03	0.35 $\pm$ 0.14	<0.001
	No.	6	10	
100 ng	Er	ND	1 $\pm$ 1	
	Ind	ND	—	
	Th	ND	6	
	No.			
10 ng	Er	11 $\pm$ 1	0 $\pm$ 0	<0.001
	Ind	+	—	<0.001
	Th	0.94 $\pm$ 0.09	ND	
	No.	7	6	

Table I Interference of delayed skin reactions to two doses of egg albumen in guinea pigs sensitized three weeks earlier

Er = mean diameter of erythema $\pm$ standard error (mm), Ind = median degree of induration, Th = mean increase in skin double thickness $\pm$ standard error (mm), No. = number of guinea pigs, ND = not done.

Skin Test		Delayed Skin Reactivity		Value of P
Antigen		Test Alone	Test Simultaneously with 100 µg of Egg Albumen	
Egg albumen 100 µg	Er	29.4 ± 1.4	ND	
	Ind	++ +	ND	
	Th	2.62 ± 0.22	ND	
	No.	14	ND	
Egg albumen 5 µg	Er	24.1 ± 1.0	14.3 ± 1.2	<0.001
	Ind	+ to ++	+	<0.001
	Th	1.97 ± 0.23	0.80 ± 0.17	<0.001
	No.	9	9	
PPD 10 µg	Er	17.0 ± 1.2	12.4 ± 0.8	<0.05
	Ind	+	—	<0.05
	Th	1.05 ± 0.29	0.39 ± 0.17	>0.05
	No.	5	5	

Table II Nonspecificity of the interference of delayed reactions to two skin tests in guinea pigs sensitized fourteen weeks earlier

Er = mean diameter of erythema ± standard error (mm), Ind = median degree of induration, Th = mean increase in skin double thickness ± standard error (mm), No. = number of guinea pigs, ND = not done.

at the early stages of immunization. The finding further shows that, in studies of the time course of delayed hypersensitivity responses, the antigen dose selected to monitor the reactivity may influence the results. Higher doses are likely to reveal an increasing sensitivity, while lower doses reach a plateau relatively early.

The simultaneous injection of 100 µg of egg albumen suppressed the delayed skin reactions to lower doses of egg albumen. This confirms the earlier similar finding with tuberculin (Baer and Kolb, 1967). The phenomenon is probably related to desensitization, the specific suppression of delayed skin reactivity after challenge with a large antigen dose (Uhr and Pappenheimer, 1958; reviewed by Jokipii, 1972). Desensitization can also be achieved by a challenge simultaneously with skin testing or a couple of hours afterwards (Uhr and Pappenheimer, 1958).

In the present study the delayed reactions to PPD were equally suppressed by the injection of egg albumen, which shows that the limiting factor is not the population of antigen-sensitive cells but rather the nonspecific amplifying mechanism. During a couple of days after desensitization, a nonspecific suppression of delayed hypersensitivity to other antigens has been observed (Uhr and Pappenheimer, 1958; Jokipii, 1970). As two simultaneous doses of 100 µg of egg albumen did not suppress each other, it seems that enough cells are available to form two infiltrates. This interpretation is supported by the recent finding that desensitized animals respond normally to exogenous skin reactive factor during the initial period of nonspecific anergy (Dwyer and Kantor, 1973).

The apparent lack of a dose response relationship in guinea pigs, which have the potential to form two strong infiltrates, may be accounted for by assuming that much more than a doubling of the stimulus is needed to produce a detectable increase in the reaction. This is supported by the finding that, although 100, 10, and 1 µg of egg albumen in separate individuals gave reactions of comparable intensity, the stimuli may have been markedly different, because the weaker reactions were suppressed, when the 100 µg was injected into the same animal.

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