

Delayed Hypersensitivity Skin Testing of 150 Volunteers

Retesting Results and Antibody Measurements

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We studied skin test reactivity to five commonly used antigens by testing 150 healthy adults. The delayed hypersensitivity (DH) skin test is widely used to assess the immune status of patients. The battery of antigens suitable for use may vary in different countries, but the reactivity to the antigens in our population did not differ remarkably from reports of other authors. The reactivity rates were: candida 32.7%, mumps 86%, streptokinase-streptodornase (SK-SD) 70%, trichophyton 0% and tuberculin 58.7%. Sixteen of the subjects were retested after 2 weeks. Only eight of them showed unchanged reactions to all five antigens. Specific IgG antibody concentrations measured by enzyme-linked immunosorbent assay (ELISA) against each of the five antigens in the serum of 42 subjects before and after testing showed great inter-individual variation. The antibody concentration did not correlate with the DH skin test results, but the testing itself increased the production of anti-mumps- and anti-SK-SD-antibodies.

Key words: antibody formation; hypersensitivity, delayed; immunocompetence; skin tests.

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The delayed hypersensitivity skin test is recommended for the assessment of immunocompetence in various clinical conditions (15). The test is technically simple and can be done *in vivo* without laboratory facilities. It is principally a measure of cell-mediated immunity, where sensitized lymphocytes react with the test antigens injected intradermally to produce an inflammatory reaction at the test site. The patients must, however, have had previous sensitization to the antigens. To be sure of a previous contact with at least some of the test antigens, a battery of common antigens is used. There may, however, be regional differences in the sensitization to the antigens generally used. Therefore, we studied the basic reactivity of a healthy Finnish population in the delayed hypersensitivity skin test to the antigens recommended by WHO (19).

It is also recommended to measure the delayed hypersensitivity skin test reaction at short intervals to get better prognostic information than from a single measurement. Therefore, we also studied the effects of repeated skin tests on the test results and, moreover, we investigated whether the test itself can produce an antibody response.

MATERIAL AND METHODS

Delayed hypersensitivity skin reactions were measured in 150 voluntary medical students and hospital employees. 55 of them were men and 95 female. Their mean age was 26 ± 7 years (range 18-56). The test subjects were in good health. 29 of them had allergic rhinitis or atopic dermatitis in their medical history, two had mild arterial hypertension and were on

diuretic treatment, two had urinary tract infections treated with sulfonamide and one had hypothyroidism treated with levothyroxin. In addition, several of the females used oral contraceptives. All the test persons had in their childhood been vaccinated against tuberculosis and most of them had suffered from mumps.

The delayed hypersensitivity reactions were tested according to the recommendations given by WHO (19). 0.1 ml of each test antigen was injected intradermally into the forearm. The test antigens were candida 1:100 (Dermatophyton-O; Hollister-Stier, Mississauga, Canada), mumps skin test antigen undiluted (Eli Lilly & Co. Ind., USA), streptokinase-streptodornase 1:100 (Varidase; Lederle, Pearl River, NY, USA), Trichophyton 1:100 (Dermatophyton; Hollister-Stier) and Tuberculin (PPD) 1 TU and 10 TU (Statens Serum Institute, Copenhagen, Denmark). PPD 10 TU was used in only 37 persons due to its excessive reactions. As a control, 0.1 ml of 0.9% NaCl solution was used. The diameters of induration were read at 24 and 48 h. An induration of > 5 mm in diameter was considered as a positive result, because this is the most commonly accepted criterion for a positive reaction (1, 3, 4, 5). The tests were carried out in the afternoons by two persons (P.N. and a nurse).

Sixteen of the volunteers were retested 2 weeks after the first intradermal injection to examine the stability of the DH skin tests. Opposite forearm to that of the first occasion was used as the second identical testing.

Antibody levels of IgG class to these five test antigens were measured in 42 subjects with an enzyme-linked immunosorbent assay (ELISA) modified from the technique described previously (11, 20). The dilutions used for attaching the antigens on to polystyrene microtitration plates (Flow Laboratories Inc., Hamden, Conn., USA) were 1:1000 for candida, 1:500 for mumps, 1:100 (10 µg/ml) for PPD, 1:100 for SK-SD and 1:100 for trichophyton. After overnight incubation at 37°C, the plates were washed and surplus binding sites on the solid-phase were saturated by incubating PBS supplemented with 1% bovine serum albumin (BSA-PBS) in the wells at 37°C for 3 h. Sera were

diluted 1:100 with BSA-PBS and incubated in the wells at 37°C for 2 h. After washes, anti-human-IgG antibodies conjugated with alkaline phosphatase (Orion Diagnostica, Helsinki, Finland) were added. After overnight incubation and washes, p-nitrophenylphosphate (Orion) was added as a substrate and the optical density was measured at 405 nm by a vertical beam photometer (Titertek/Multiscan, Eflab Oy, Espoo, Finland). The concentration of antibodies in the sample was expressed as relative units (EIU), where one unit was 1/100 of the corresponding antibody concentration in the reference serum. (Anti-PPD and anti-candida antibody reference sera were those used in routine measurements of these antibodies in our laboratory. When levels of anti-mumps, anti-SK-SD and anti-trichophyton antibodies were measured in each case a serum belonging to the present material was used as a reference.)

The measurements were made 10 days before and 1 week after the first injection. The antibody response was also measured in connection with the retesting procedure in 16 persons, whose antibody responses were already measured during the first test and then 1 week after the second injection. The serum antibody concentrations were also measured in eight persons who had received the first but not the second injection.

Changes in serum IgG antibody concentrations were studied by the Student's *t* test and by the paired *t* test. Correlations between the skin test results and antibody concentrations were studied by linear regression analysis.

RESULTS

The number of positive DH reactions to each antigen is given in Table 1. The reactivity to mumps antigen was common, altogether 129 subjects (86%) had a positive response. Reactivity rate to SK-SD antigen was also high (70%) and the mean diameter of indurations was largest with this antigen (10.7 mm). None of the 100 subjects reacted to trichophyton antigen. Numbers of positive results with PPD

and candida antigens were slightly lower than expected; 58.7% and 32.7% respectively.

Five subjects (3.3%) did not react to any of the five antigens (Table 2), 26 subjects (17.3%) had only one positive result, but nearly 80% of the volunteers reacted to at least two antigens.

Table 1
Number of positive reactions (induration ≥ 5 mm) in delayed hypersensitivity skin tests

	Pos. results	Percent	No. of subjects
Candida	49	32.7	150
Mumps	129	86	150
PPD	88	58.7	150
SK-SD	105	70	150
Trichophyton	0	0	100
0.9% NaCl	0	0	150

Table 2
Number of persons reactive to 0, 1, 2, 3, 4 or 5 antigens (candida, mumps, PPD, SK-SD, trichophyton)

No. of antigens	No. of reactive persons	Percent
0	5 (anergic)	3.3
1	26	17.3
2	41	27.3
3	57	38
4	21	14
5	0	0

Table 3
Delayed hypersensitivity skin test results in 16 retested volunteers

	Positive results in the first- and second test		Alterations in the second test:			
			Re-mained +	Turned + → -	Turned - → +	
Candida	3	6	3	0	3	
Mumps	15	14	14	1	0	
PPD	9	8	7	2	1	
SK-SD	13	13	12	1	1	
Trichoph.	-	-	-	-	-	
0.9% NaCl	-	-	-	-	-	

When 16 volunteers were retested 14 days after the first test, at least one skin test result had changed in eight subjects. Only the unreactivity to trichophyton and to the control NaCl-solution remained unchanged (Table 3).

The serum IgG-antibody background levels measured before the injections of the skin test antigens did not correlate with the skin test results. The concentrations of anti-mumps-antibodies increased significantly from the first to the second measurement ($P < 0.001$) (Table 4).

Table 4
Serum IgG-antibody concentrations (percent of reference serum) before, and 1 week after, the delayed hypersensitivity skin test in 42 volunteers. Means ± SD

	Before	After
Anti-candida	34.3 ± 68	58.1 ± 80
Anti-mumps	44.4 ± 23	69.3 ± 26***
Anti-PPD	4.3 ± 6	4.0 ± 5
Anti-SK-SD	27.7 ± 30	33.5 ± 35
Anti-trichophyton	37.8 ± 29	37.8 ± 24

*** $P < 0.001$.

Table 5
Serum IgG-antibody concentrations (percent of reference serum) in 16 retested persons. The values of the eight persons without the second injection are in parenthesis. Means ± SD

	Before	Week after first injection	Week after second injection
Anti-candida	39.3 ± 97 (40.4 ± 26)	41.6 ± 94 (44.6 ± 26)	41.8 ± 42 (35.6 ± 27)
Anti-mumps	42.2 ± 25 (52.6 ± 14)	68.9 ± 28 (70.0 ± 24)	63.9 ± 32 (61.6 ± 27)
Anti-PPD	6.2 ± 8 (3.4 ± 5)	4.1 ± 4 (3.4 ± 5)	3.7 ± 4 (2.7 ± 3)
Anti-SK-SD	23.4 ± 19 (34.8 ± 55)	32.5 ± 35 (21.0 ± 19)	50.1 ± 48** (40.5 ± 48)
Anti-trichoph.	29.1 ± 25 (38.7 ± 36)	31.2 ± 19 (43.5 ± 40)	31.1 ± 17 (32.1 ± 35)

** $P < 0.01$.

We were unable to find a correlation between the antibody levels and the skin test results at the second measurement either. Anti-SK-SD-antibody levels increased significantly after the second antigen injection ($P < 0.01$), but a slight increase (N.S.) in the mean of antibody concentration was detected even in the controls, who did not get the second injection (Table 5). A great variation in the antibody levels between individuals was observed.

DISCUSSION

Our subjects were healthy volunteers, because we wanted to test the battery of recall antigens in a population with no expected defects in their cell-mediated immune mechanisms or in their inflammatory responses. In our material only 3.3% failed to react to any of the five antigens. This frequency of anergy is acceptable since we can hardly reach 100% reactivity with any battery of antigens in a large population.

The antigens we used are those recommended by WHO. These antigens are widely used in approximately the same concentrations in North America (2, 4, 5, 12) and Europe (1, 3, 17). In Finland practically all adults have suffered from mumps and about 90% of the population has been BCG vaccinated, thus explaining the high reactivity to these antigens in this study the recommended 10 TU PPD dose caused two excessive reactions at the start of our study the recommended 10 TU dose PPD caused two excessive reactions at the start of our series. Therefore, we had to omit this dose and use only a 1 TU PPD dose. Reactivity to candida was lower (32.7%) in our material than in an earlier study from Finland with 77% reactivity (8). In the latter study a self-made, partially purified candida antigen was used instead of the commercial antigen used in our study. The high reactivity to SK-SD in this study as in studies elsewhere can be explained by the high frequency of streptococcal infections overall. In general, the antigens and antigen doses we used equalled those of other authors and our reactivity rates resemble those reported by them (1, 5, 12, 17). Exceptions were the lower

reactivity to candida antigen in our study and absence of reactions to trichophyton antigen.

In our study trichophyton antigen never caused even the slightest erythema. Since there were anti-trichophyton-antibodies in the serum of our subjects, they had had earlier contact with trichophyton fungi. So the absence of positive reactions was due most probably to a weak preparation. Other authors have reported similar experiences (17).

The results of DH skin tests are often graded according to the number of positive reactions when several antigens are used (1, 2, 5, 17). This classification of patients as more or less reactive may be informative in large materials (4, 5), but it does not function at the individual level, because one cannot be sure that the testee has been reported to cause a booster effect (2, 9, 12), but even a suppressive effect in the test results only to classify subjects into anergic or reactive.

Repeated skin testing with candida, mumps, SK-SD, trichophyton and tuberculin antigens has been reported to cause a booster effects (2, 9, 12), but even a suppressive effect in connection with repeated injections with candida, mumps and tuberculin antigens has been reported (14, 18). In our study reactions to candida showed a slight booster phenomenon, but in general the DH skin test results were quite stable when these were repeated 2 weeks later. The changes noted in skin test results were unmistakable without any borderline cases. Nevertheless, we recommend the use of a control group in connection with repeated DH skin tests.

Few studies investigating the effects of DH skin testing on antibody production have been made. Saxon et al. (13) have described a decrease in tetanus antibody production when re-stimulation with tetanus was used. Lesourd et al. (10) reported increased tetanus, but decreased tuberculin antibody production after testing with seven skin test antigens. Even these single changes were of short duration. We did not find any correlation between the skin test results and antibody concentrations. If the strength of skin reactions in these tests is mainly dependent on macrophages and T lymphocytes, the overall ac-

tivity of these is not in relation to corresponding serum IgG antibody concentrations. Skin testing with candida, trichophyton, or tuberculin antigens did not cause any significant changes in the corresponding antibody production. Mumps, the only viral antigen we used, caused a significant increase in anti-mumps-antibodies measured 1 week after injection. The SK-SD-antigen also caused an increase in corresponding antibodies, but this change was not detected until 3 weeks after the first injection. Structural differences in antigens may explain the divergence in the effects on antibody production.

When DH skin tests are used the limitations of the test on the individual level must be realised. The test does not measure the primary events in defence against infections, but, however, the changes in the skin test results probably run parallel with changes in parameters connected with early stages of host defence, e.g. activity of lymphokines (16). The vague results of repeated testing and our unexpected results in antibody measurements indicate that although DH skin tests are widely used for different purposes, there are still unsolved problems related to them.

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