Delayed Skin Hypersensitivity in Man. The Effect of Repeated Tests with Low-dose Antigen

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Merk K, Björkholm M, Holm G, Mellstedt H. Delayed skin hypersensitivity in man. The effect of repeated tests with low-dose antigen. Acta DermVenereol (Stockh) 1988; 68: 450–453.

The effects of repeated applications of low doses of antigen on subsequent delayed cutaneous hypersensitivity (DCH) responses induced by the same antigen were evaluated. Fourteen healthy individuals were tested with seven different antigens given intracutaneously with the Multitest system device (Institute Mérieux). The procedure was repeated on four occasions at 2-week intervals. At the first test, 11 and 13 participants displayed DCH to tetanus and tuberculin, respectively. Neither the proportion of responding individuals nor the mean diameter of the reactions was changed in subsequent tests. However, the DCH pattern varied significantly between subjects ($p<0.001$; variance analysis). There was also a significant variation between the test occasion and skin reactivity. It is concluded that repeated applications of low-dose antigen by Multitest do not boost the DCH response. However, because of the low frequency of response to certain antigens and the low reproducibility of the test, the usefulness of this test system to evaluate DCH reactivity is limited. (Received February 11, 1988.)

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Tests for evaluation of cell-mediated immunity are required in studies on patients with immunodeficiencies. Delayed cutaneous hypersensitivity (DCH) following intradermal injections of recall antigens has been used to explore the immune state as a predictor of prognosis in cancer patients (1–3) and even in normal subjects (4). DCH has also been used to monitor the in vivo effects of immunostimulating agents (5) and the efficacy of various treatment modalities in cancer patients (6). In the latter situation, however, the interpretation of repeated tests may be hampered by boosting of the immune reactivity by previous tests (7, 8). Such unwanted effects could be avoided or diminished by the use of low doses of antigen. This can be achieved by the recently developed Multitest system, which allows the application of small doses of seven different antigens intracutaneously.

We have previously shown that a single test with this system did not influence lymphocyte subpopulations or in vitro lymphocyte functions as studied before and during the first month following the test (9). In this study we have evaluated the effects of repeated cutaneous applications of low doses of seven antigens on subsequent DCH responses induced by the same antigens.

MATERIAL AND METHODS

Subjects

Fourteen healthy members (8 males and 6 females) of the hospital staff were studied. Their mean age was 38 years (range 25–59). No participants had signs of infections and their ESR was normal during the study period. None was receiving medication.

Skin testing

The multitest system (Institute Mérieux, Lyon, France) was used. It consists of a plastic disposable multipuncture device by which low concentrations of seven glycerinated antigens (Tetanus, Diphtheria, Streptococcus, Tuberculin, Candida, Trichophyton and Proteus) and a control substance (70% glycerol solution) can be administered. All skin tests were applied to the volar surface of the subject's

forearm by the same investigator, who also read and recorded the responses after 48 h. Each subject was tested on five occasions at two 2-week intervals. An induration of 2 mm was considered a positive reaction.

Statistics

To identify the variable(s) of greatest importance with regard to skin reactivity, a two-way analysis of variance was used.

RESULTS

Most of the participants showed a DCH response to tetanus and tuberculin in test I (Table I). Positive reactions to diphtheria, streptococcus and candida were seen in approximately half the tested subjects, while only one person was sensitized to trichophyton and proteus. However, neither this difference nor changes in the response to other antigens reached statistical significance. The number of subjects responding to candida antigen increased from 6 to 11. Moreover, some subjects who became DCH-positive after one or more tests, showed a negative reaction in later tests. The fraction of candida-responding cases was not reflected in an increase in the mean skin induration (Fig. 1).

The mean diameter of responses to each of the antigens in five consecutive tests is shown in fig. 1. No significant time-related changes in DCH reactions were detected. The relatively high mean diameter of the induration induced by tetanus antigen in weeks 4 and 8 is explained by the reactivity of one individual after repeated skin tests.

Variance analysis showed a highly significant variation in the skin reactivity between subjects ($p<0.0001$). The variation of the cutaneous response to tuberculin for each participant on different occasions (subject period interaction variation) was significant ($p<0.001$), as were subject antigen interaction and the variation between different antigens ($p<0.001$). Though the variation between time (period) of the test was less pronounced than the other variables tested, it did reach statistical significance ($p<0.05$).

Table I. *DCH responses in 14 normal volunters after multiple antigen skin tests*

	Test I (week 0)		Test V (week 8)	
Antigen	No pos skin tests/ total	Mean diameter (mm) range	No pos skin tests/ total	Mean diameter (mm) range
Tetanus	11/14	4.1 0–10.5	11/14	5.5 0–35
Diphtheria	7/14	2.1 0–10	6/14	1.9 0–8.5
Streptococcus	8/14	2.4 0–6.5	5/14	1.2 0–5
Tuberculin	12/14	7.5 0–13	13/14	6 0–10
Candida	6/14	1.5 0–5	11/14	2.7 0–6
Trichophyton	1/14	0.5 0–7	3/14	0.6 0–5
Proteus	1/14	0.1 0–2	4/14	0.7 0–3.5

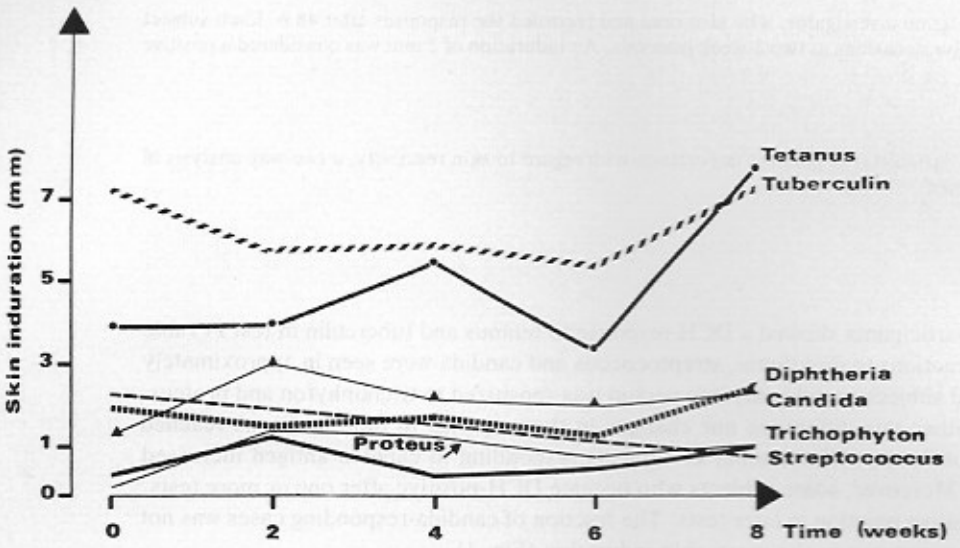

Fig. 1. Effect of serial skin testing with multiple antigens on specific DCH reactivities. The mean diameters of induration (in mm) of 14 subjects tested on five occasions.

DISCUSSION

The incidence of cutaneous DCH reactions to seven recall antigens in this series of volunteers was rather lower than that previously reported using the Multitest device (9, 10). Very few responders to trichophyton and proteus antigens were noted. Thus, these antigens are of no use for the immunological evaluation of Swedish patient populations.

In spite of occasional DCH enhancement, no significant boosting effect of repeated application of the antigens was observed, as has also been described by another group (10). However, the value of the test can be questioned due to the low frequency of responders to certain antigens in a Swedish population and the overall low reproducibility of the test.

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REFERENCES

1. Björkholm M, Holm G, Mellstedt H. Immunocompetence in patients with Hodgkin's disease. In: Lacher M, Redman J, Washington; eds. Consequences of survival in Hodgkin's disease. Lea & Febiger, 1988 (in press).
2. Pietsch JB, Meakins JL, MacLean LD. The delayed hypersensitivity response: Application in clinical surgery. Surgery 1977; 182: 349–355.
3. Pinsky CM, El Domeiti A, Caron AS, Knapper WH, Oettgen HF. Delayed hypersensitivity reactions in patients with cancer. Recent Results Cancer Res 1974; 47: 37–41.
4. Roberts-Thomson IC, Whittingham S, Youngchaiyud U, MacKay IR. Ageing, immune response and mortality. Lancet 1974; ii: 368–370.
5. Lang JM, Giron C, Oberling F, Marchiani C, Zalisz R. Conversion of skin tests in cancer patients after a short course of treatment with a new immunostimulating compound. Cancer Immunol Immunother 1980; 8: 273–274.

6. Daly JM, Dudrick SJ, Copeland EM. Intravenous hyperalimentation: Effect on delayed cutaneous hypersensitivity in cancer patients. Ann Surg 1980; 192: 192–592.
7. Hogan TF, Borden EC, Freeberg BL, Vieau J, Fang-Yuh H, Crowley J. Enhancement in recall antigens responses by frequent repetitive skin testing with Candida, Mumps and Streptokinase–streptodornase in 38 normal adults. Cancer Immunol Immunother 1980; 10: 27–31.
8. Keystone EC, Demerieux P, Gladman D, Poplonski L, Piper S, Buchanan R. Enhanced delayed hypersensitivity skin test reactivity with serial testing in healthy volunteers. Clin Exp Immunol 1980; 40: 202–205.
9. Björkholm M, Holm G, Mellstedt H. No effect of delayed cutaneous hypersensitivity testing (Multitest) on blood lymphocyte counts and functions. Acta Med Scand 1983; 214: 399–401.
10. Lesourd BM, Winters WD. Specific immune responses to skin test antigen following repeated multiple antigen tests in normal individuals. Clin Exp Immunol 1981; 50: 635–643.

Nickel Dermatitis and Diet: Clinical Improvement and a Reduction in Blood and Urine Nickel Levels with a Low-nickel Diet

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Gawkrodger DJ, Shuttler IL, Delves HT. Nickel dermatitis and diet: Clinical improvement and a reduction in blood and urine nickel levels with a low nickel diet. Acta Derm Venereol (Stockh) 1988; 68: 453–456.

A 27-year-old nickel-sensitive female who had had continuous spontaneous flare-ups of eczema, including at sites of previous metal contact, experienced a clearing of her eruption after commencing a low-nickel diet. When on the diet, whole-blood and urinary nickel levels fell to half or less of pre-diet values and this coincided with the clinical improvement. Low-nickel diets should be considererd for patients who are highly nickel sensitive. *Key words: Nickel sensitivity.* (Received February 19, 1988.)

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Orally ingested nickel can cause a flare-up of dermatitis in nickel-sensitive subjects (1). The amount of nickel required is usually much greater than the normal daily intake (2), although some patients might react to smaller quantities. About a half of nickel-sensitive patients improved on a low-nickel diet (3, 4); urinary nickel levels may fall, but not always by very much (3). However, the rationale for the diet has been challenged because only large amounts of orally administered nickel cause flare-ups (5) and diets are hard to assess objectively on a double-blind basis. We have treated a highly nickel-sensitive subject with a low-nickel diet and measured her blood and urinary nickel levels to provide objective data on the pharmacological effect of the diet.

PATIENT, METHODS AND RESULTS

A 27-year-old woman reported a 5-year history of reacting to jewellery and metal studs on clothing. The problem began 9 months after having her ears pierced. Over the 2 years before presentation the eczema worsened and would appear spontaneously at sites previously involved, without further local contact. She gave no history of atopy. Patch testing revealed a 3+ reaction at 48 h and 120 h to 5% nickel sulphate in petrolatum, but not to any other allergen in the standard battery.

She commenced a strict low-nickel diet similar to that outlined by Veien & Andersen (6): she ate fish, fruit, cheese, bread and crackers, drank beer and lager but avoided all tinned products, peanuts, bananas and chocolate. She took reduced amounts of vegetables and dairy products and used no