

Cell-mediated immunity assessed by skin testing (Multitest[®])

II: Correlation between responses from arm and back

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In 60 healthy adult volunteers and 58 patients with gastrointestinal disease a test system (Multitest[®]) consisting of a plastic disposable multiple-puncture device capable of simultaneously applying seven delayed-type hypersensitivity antigens and a glycerin/saline diluent (negative control) was assessed. The Multitest device was applied on both the inner side of the forearm and on the back for assessment of cell-mediated immunity (CMI). The antigens used were two toxoids, tetanus and diphtheria, three bacterial antigens, Streptococcus, tuberculin and Proteus and two fungal antigens, Candida and Trichophyton. A scoring system based on both number and size of positive response revealed a median "score" on arm and back of 19 mm and 14 mm respectively, in the healthy volunteers and a median "score" of 12 mm and 8 mm respectively in patients with gastrointestinal disease. In both groups a significant difference was found between back and arm ($P < 0.01$). The coefficient of determination (r^2) shows that only 64 % of the variability in scores on the back is explained by the regression line. Therefore, scores obtained from tests on the back cannot be interpreted with reference to normal values originating from tests applied to the inner side of the forearm

Key words: cell-mediated immunity; delayed hypersensitivity; skin testing.

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In healthy Danish adults normal values have recently been reported for cell-mediated immunity assessed by skin-testing with the Multitest[®] system (8). Impaired cell-mediated immunity assessed by skin testing has been claimed to identify patients with increased risk of post-operative complications (7, 9, 11). However, in patients coming to surgery, especially patients at risk, the forearms are often less suitable for skin testing due to phlebitis or oedema following preoperative parenteral nutrition, blood sampling, etc. In such cases sites for skin testing other than the forearms would be desirable, for example the back.

In previously published papers normal values in healthy study groups refer to responses from tests applied to the inner side of the forearm (2,

3, 4, 10). This paper presents a comparison of the responses to seven antigens simultaneously applied to the inner forearm and the back in 118 adults.

MATERIAL AND METHODS

Sixty healthy adult volunteers and 58 adult patients with gastrointestinal disease were tested. Subjects with a history of any type of allergy were excluded in both groups. The healthy volunteers (32 men and 28 women) had a median age of 54 years (range 21-77). The group of patients comprised 34 men and 24 women with a median age of 58 years (range 27-79).

Informed consent was obtained from all participants, and the study protocol was approved

by the regional ethical committee and the National Health Board.

The test was applied to the inner side of the forearm, and on the back between the spin and scapula. Testing and reading of responses were performed by the authors. Testing procedure and antigens have previously been described (8). The cutaneous response to each antigen was read at 48 h, and expressed as the median diameter of the two perpendicular diameters.

Measurements, taken with a caliper-compass and expressed in millimeters, allow the following definitions: 1) a positive reaction is a median diameter ≥ 2 mm, 2) anergy is the absence of positive reactions for all of the antigens, and 3) the "score" of the subject under study is the

sum in millimeters of all the median diameters of positive reactions. This "score" enables an overall quantification of cell-mediated immunity of the individual.

RESULTS

Table 1 shows the percentage of incidence of positive cutaneous responses to individual antigens. In both healthy volunteers and patients a smaller number of positive responses was found on the back compared to the arm. A significant difference was found between the number of positive reactions on the arm and back with regard to the antigens from Tetanus, Diphtheria, Streptococcus, tuberculin and Candida.

Table 1
Percentage of incidence of positive cutaneous responses^a to seven antigens in 118 adults

Antigen	Healthy volunteers		Patients	
	Arm (n = 58)	Back	Arm (n = 60)	Back
Tetanus	53	*	45	30
Diphtheria	61	*	56	49
Streptococcus	40	*	32	26
Tuberculin	84	*	78	70
Candida	53	*	44	35
Trichophyton	9	6	8	7
Proteus	15	10	11	8

^aPositive responses are ≥ 2 mm induration at 48 h

* $P < 0.01$ (Wilcoxon's test)

Table 2

Median diameters of induration and range in positive^a cutaneous responses to seven antigens and median score and range^b.
Number of positive responses to each antigen appears from Table 1

Antigen	Healthy volunteers		Patients	
	Arm (n = 58)	Back	Arm (n = 60)	Back
Tetanus	4 (2-16)	3 (2-12)	4 (2-14)	3 (2-10)
Diphtheria	4 (2-14)	3 (2-11)	4 (2-10)	3 (2-10)
Streptococcus	3 (2-8)	3 (2-6)	3 (2-6)	3 (2-6)
Tuberculin	6 (2-24)	5 (2-20)	5 (2-18)	4 (2-20)
Candida	3 (2-10)	2 (2-8)	3 (2-7)	2 (2-6)
Trichophyton	3 (2-6)	3 (2-4)	3 (2-5)	2 (2-4)
Proteus	3 (2-5)	3 (2-4)	3 (2-4)	2 (2-5)

^aPositive responses are ≥ 2 mm induration at 48 h

^bThe "score" is the sum in mm of all median diameters in a person

* $P < 0.01$; ** $P < 0.05$ (Wilcoxon's test)

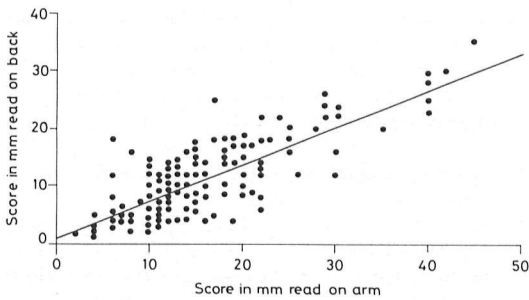


Fig. 1. Scatter diagram of "scores" from back and arm with regression line.

Correspondingly, Table 2 shows a significantly smaller diameter of positive responses on the back in regard to the same antigens except *Streptococcus* antigen. The median differences in "score" between arm and back were highly significant.

In Fig. 1 the corresponding "scores" from arms and backs are shown in a scatterplot. The correlation coefficient is 0.81 and the regression equation is "back" = $1.03 + 0.64 \times \text{"arm"}$, the slope being significantly different from zero ($P < 0.05$) in contrast to the intercept ($P > 0.05$).

DISCUSSION

Only 64% of the variability in scores on the back is explained by the regression line. Furthermore, for individual cases there may be considerable imprecision if the regression equation is used to predict scores on the arm from scores on the back. Consequently, scores obtained from a test on the back cannot be interpreted with reference to normal values originating from tests applied to the inner side of the forearm.

In general, skin reactions depend on the nature and quantity of antigens presented, the sensitization of the individual and local skin factors. In the present study all factors except the test region were constant, and compared with the forearm the skin on the back was found to be less sensitive to superficially inoculated microbial antigens. Our findings contrast with

studies of similar delayed hypersensitivity reactions to patch testing with contact allergens and irritants (5). Here the strongest responses were found on the upper part of the back and this was presumed to be due to pressure of clothes and the weight of the body on deposited allergens when lying down. In the present study no allergic material was left on the skin surface. Local factors such as pressure and concentration of sebaceous glands would therefore be of minor importance for the absorption and subsequently for the size of the allergic reaction. This indicates that the regional variation is highly influenced by the test procedure. As in the patch test study, results from skin prick tests investigating immediate reaction to allergens (i.e. reaction within 15 min) have shown the skin on the back to have a larger reaction compared with the arms (1). Since mechanisms for immediate and delayed skin reaction are different, relative diversities in sensitivity of the skin in different regions are not contradictory. The immediate skin reaction is largely dependent on the density of immunoglobulin E-carrying mast cells in the test area, which release mediators thereby creating local oedema. Since these cells vary considerably in number, immediate reactions to the same substance may be different on different areas of the inner side of the forearm (12, 13). The delayed reactions, on the other hand, involve both local infiltration of cells from the blood vessels and mediators from these cells. As shown in the present study such reactions would be expected to diminish in regions where vascularization is less abundant as seen in the back with its subcutaneous fat layer.

Previously published reference values originate solely from tests applied to the inner side of the forearm (2, 3, 4, 6, 8). We therefore concluded that in surgical or other patients the back cannot be used as test site if results are to be compared to reference values from a normal population.

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