

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

I. Normal values in healthy Danish adults

F. MOESGAARD,¹ M. LYKKEGAARD NIELSEN,¹ P. NØRGAARD LARSEN,¹ S. CHRISTOPHERSEN¹ & H. MOSBECH²

¹Department of Surgical Gastroenterology C, and ²Department of Medicine TTA, Allergy Unit, Rigshospitalet, University Hospital, Copenhagen, Denmark

The Multitest CMI system consists of a disposable multiple puncture device that simultaneously applies seven standardized recall antigens for assessment of cell-mediated immunity (CMI). The seven antigens included were toxoid from *Clostridium tetani* and *Corynebacterium diphtheriae*, tuberculin, plus antigens from streptococcus (group C), *Candida albicans*, *Trichophyton mentagrophytes*, and *Proteus mirabilis*. A population of 352 healthy Danish adults, aged between 17 and 90 years, was tested to determine the incidence and size of delayed-type hypersensitivity (DTH) responses. All but six healthy adults (98 %) responded to one or more antigens, the median number of positive responses being four in males and three in females. The incidence of positive responses ranged from 91 % for tuberculin to 11 % for trichophyton. The number of positive responses declined with age, being somewhat faster in females than males. Six of the seven antigen response rates were significantly lower in the over 65-years-olds, the only exception being trichophyton, and for four of the seven antigens significantly lower in females compared to males. When correlated to age and sex no major differences were found with regard to the size of response to the seven antigens except that the tuberculin response was larger in males. A scoring system based on both number and size of positive responses revealed significant age and sex related differences. The median "score" in 17-65-year-old males and females were, respectively, 17 mm and 14 mm compared to 13 mm and 8 mm in those over 65 years old ($P < 0.001$ for both comparisons).

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

Accepted for publication 17 April, 1987

Until recently, testing for delayed cutaneous hypersensitivity with multiple antigens was time-consuming, the standardization of testing techniques and subsequent interpretation of reactions often being troublesome. A newly introduced system (Multitest[®]), using an applicator with eight heads, permits rapid simultaneous intradermal application of seven antigens (6). Investigations of healthy volunteers from France, Switzerland, UK and USA have shown different reference values for several of the antigens used (7, 8, 10, 13).

Infectious complications following gastroin-

testinal surgery depend on the balance between host-defence mechanisms and intraoperative contamination. Cutaneous delayed-type hypersensitivity (DTH) reactions to intradermally injected recall antigens reflect cell-mediated immunity (CMI), and depressed CMI indicated by impaired DTH has been claimed to identify those patients with an increased risk of surgical infections (12, 14).

It was the aim of the present study to provide regional values for delayed hypersensitivity measured by the Multitest system. This paper presents the number and size of reactions to

seven antigens applied by the Multitest system in 352 healthy adult Danish volunteers.

MATERIAL AND METHODS

Study population

352 clinically healthy adult volunteers in the greater Copenhagen area were tested. They were all white, and subjects under the age of 65 years were employees in offices or factories. Subjects aged 65 years or older were either still employed or residing at retirement homes. The offices, factories, and retirement homes in question were chosen at random. On entering the study all subjects were free of any chronic disease. Volunteers with a history of any acute disease within 3 months of the study or with a history of any type of allergy were excluded. Beforehand it was decided to place the volunteers into six age group categories as shown in Table 3. Additionally, results were calculated for two large age groups (cf. Fig. 1): 17–65 years and the over 65-year-olds. In the first group of 224 subjects there were 115 men with a median age of 39 years and 109 women with a median age of 33 years. The older group comprised 128 subjects: 59 men with a median age of 76 years (range 66–90) and 69 women with a median age of 77 years (range 66–90). In both groups, the age difference observed between men and women was not significant.

Informed consent was obtained from all participants, and the study protocol was approved by the regional ethical committee and the National Health Board.

Testing procedure, antigens, and reading of responses.

All testing and reading of responses were performed by the authors. Testing procedure, antigens and reading of responses have previously been described in detail (6, 8). Briefly, the test is performed on the inner side of the forearm by means of a multipuncture system, Multitest (Institute Mérieux, Lyon, France). The Multitest is a methyl polymetacrylate applicator with eight heads each with nine tines, laid out opposite each other and loaded with sterile anti-

genic solutions. Simultaneous introduction of the antigens intradermally is ensured by applying pressure for approximately 5 s. Antigenic extracts are prepared from inactivated culture supernatants and stabilized by a glycerine solution 70% weight/vol. Seven antigens are used: toxoid from *Clostridium tetani* and *Corynebacterium diphtheriae*, tuberculin, plus antigens from streptococcus (group C), *Candida albicans*, *Trichophyton mentagrophytes*, and *Proteus mirabilis*. The eighth head carries only glycerine and is thus used as a negative control. Each antigen solution is chemically and biologically standardized. The antigenic potency is comparable for each batch as proven previously by tests of delayed hypersensitivity activity in the guinea pig according to the officially recommended Code of Federal Regulation (15). The means of determining the optimal antigenic concentration for investigating a normal adult as well as details concerning the activity of these antigens have been described in previously published papers (6, 15).

Cutaneous reactions are read at 48 h to evaluate delayed hypersensitivity reactions. Determination of the diameter, corresponding to the median value of two perpendicular diameters allows the reaction to be assessed quantitatively. These measurements are taken with a caliper-compass and are expressed in millimeters. The following definitions are used: 1) A positive reaction is a median diameter ≥ 2 mm. 2) Anergy is the absence of positive reactions for all of the antigens. 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.

Calculations

Positive responses of each of the antigens and score were compared with regard to sex and age by the Mann-Whitney test. A P -value < 0.05 was considered significant.

RESULTS

The incidence percentage of positive cutaneous responses to individual antigens is shown in

Table 1

Incidence percentage of positive cutaneous responses* to seven antigens in 352 healthy adult volunteers

Antigen	Age groups in years		Significance of difference	Sex		Significance of difference
	17-65 (n = 224)	> 65 (n = 128)		Males (n = 174)	Females (n = 178)	
Tetanus	61	27	$P < 0.001$	58	41	$P < 0.001$
Diphtheria	75	61	$P < 0.001$	70	69	NS
Streptococcus	38	18	$P < 0.001$	28	34	NS
Tuberculin	96	77	$P < 0.001$	95	83	$P < 0.001$
Candida	73	56	$P < 0.002$	73	61	$P < 0.05$
Trichophyton	12	9	NS	13	8	NS
Proteus	25	9	$P < 0.001$	24	15	$P < 0.05$

* Positive responses are ≥ 2 mm induration at 48 h.

Table 1. The overall response rates for all volunteers varied from 91 % for tuberculin to 11 % for trichophyton. When related to age, the response rate for each of the seven antigens was lower in those over 65 years, and significantly so for six of seven antigens. The incidence of positive reactions to each antigen showed no significant difference between males and females for three of the antigens, i.e. diphtheria, streptococcus, and trichophyton. The incidence was significantly higher in males for the other four antigens.

The median diameters of induration in positive responses are seen in Table 2. Regarding age there were no significant differences between younger volunteers and those over 65 years ($P > 0.05$ for all comparisons). Median

diameters in positive responses showed no significant differences between the sexes for six of the antigens ($P > 0.1$ for all comparisons), whereas the size of reaction for tuberculin was significantly larger in males ($P < 0.0001$).

Cumulative response rates to the seven antigens are found in Table 3. All volunteers between 17 and 65 years of age reacted to at least one antigen. Six of 128 volunteers (4.7 %) over the age of 65 years did not react to any of the seven antigens (anergic subjects). A decline in the cumulative incidence of positive responses was observed in all age groups, but was more pronounced in the older than in the younger persons. The decline in response rates to two or more antigens was somewhat faster for females than males. The median number of positive responses was four in males and three in females.

The cumulative incidence of "score" is seen in Table 4. The fifth and 95th percentiles for each of the six age groups can be read from the table. For all ages the fifth and 95th percentiles were, respectively, 2-4 mm and 25-29 mm. The corresponding percentiles for males were, respectively, 5-9 mm and 30-34 mm, and for females 2-4 mm and 20-24 mm.

Median "scores" with interquartile ranges for males and females divided into the two age groups are shown in Fig. 1. The median "score" of 17 mm in 17-65-year-old males was significantly higher than the median "score" of 13 mm in males over 65 years ($P < 0.001$). A similar and significant difference ($P < 0.001$)

Table 2

Median diameter and range (mm) of induration in positive* cutaneous responses to seven antigens in 352 healthy adult volunteers. Number of positive responses to each antigen appears from Table 1

Antigen	Age groups in years		Sex	
	17-65	> 65	Males	Females
Tetanus	4 (2-18)	4 (2-10)	4 (2-18)	4 (2-16)
Diphtheria	3 (2-15)	4 (2-15)	3 (2-16)	3 (2-15)
Streptococcus	3 (2-6)	3 (2-5)	3 (2-5)	3 (2-6)
Tuberculin	6 (2-30)	6 (2-19)	7 (2-21)**	5 (2-30)
Candida	3 (2-8)	3 (2-9)	3 (2-9)	3 (2-6)
Trichophyton	3 (2-5)	3 (2-4)	3 (2-8)	3 (2-9)
Proteus	2 (2-5)	3 (2-4)	2 (2-5)	2 (2-3)

* Positive responses are ≥ 2 mm induration at 48 h.

** $P < 0.001$.

was found between younger and older females with median “scores” of 14 mm and 8 mm, respectively. The difference in median “score” between males and females was significant in the 17–65-year-olds, in those over 65 years old, and when all ages were compared ($P < 0.001$ for all three comparisons).

DISCUSSION

Part of our anti-infectious defence mechanisms are cell-mediated with sensitized T lymphocytes as effector cells. For the measurement of various elements in cell-mediated immunity several

more or less indirect *in vitro* methods are available, such as macrophage migration inhibition test, lymphoblast transformation test and measurement of T lymphocyte subpopulations or lymphokines. These methods require special laboratory facilities as well as highly qualified staff, and several days will usually elapse before the results are obtained. *In vivo* techniques measuring the immunisation capacity of an individual by means of new antigens such as di-nitrosobenzene take an even longer period before results can be read. Furthermore, these tests can only be performed once with each antigen, and, in general, sensitization to new antigens

Table 3
Cumulative incidence of positive cutaneous responses* to seven antigens in 352 healthy adult volunteers

Age group (years)	Number tested	Anergic subjects (%)	Per cent of subjects positive to number of antigens						
			≥ 1	≥ 2	≥ 3	≥ 4	≥ 5	≥ 6	7
17–25	61	0	100.0	98.7	93.4	70.5	37.7	9.8	0
26–40	71	0	100.0	98.6	91.6	67.4	28.8	5.6	0
41–65	92	0	100.0	81.5	48.9	21.3	5.4	2.2	0
66–75	57	3.5	96.5	89.5	63.2	24.4	1.7	0	0
76–85	55	3.6	96.4	78.2	54.5	20.0	5.4	3.6	1.8
> 86	16	12.5	87.5	68.8	31.3	18.7	12.5	0	0
All ages	352	1.7	98.3	92.9	76.1	46.6	19.6	4.8	0.9
Males	174	1.2	98.8	97.7	82.1	52.9	27.0	6.9	1.7
Females	178	2.3	97.7	88.2	70.2	40.5	12.4	2.8	0

* Positive responses are ≥ 2 mm induration at 48 h.

Table 4
Cumulative incidence of “score” of seven antigens in 352 healthy adult volunteers. The “score” is the sum in mm of all median diameters of positive reactions

Scores (mm)	Age groups in years						All ages (<i>n</i> = 352)	Males (<i>n</i> = 174)	Females (<i>n</i> = 178)
	17–25 (<i>n</i> = 61)	26–40 (<i>n</i> = 71)	41–65 (<i>n</i> = 92)	66–75 (<i>n</i> = 57)	76–85 (<i>n</i> = 55)	> 86 (<i>n</i> = 16)			
< 2*	0	0	0	3.5	3.6	12.5	1.7	1.2	2.3
2–4	1.6	1.4	1.0	8.7	10.9	31.2	5.4	1.2	9.6
5–9	9.8	11.2	8.7	22.8	49.0	81.2	21.3	10.3	32.0
10–14	36.0	42.0	40.2	59.6	76.3	81.2	50.6	37.9	62.9
15–19	72.1	71.8	70.6	87.7	94.5	93.7	78.7	71.3	85.9
20–24	91.8	90.1	88.0	96.4	96.3	93.7	92.0	88.5	95.5
25–29	96.7	94.3	93.4	94.4	96.3	100.0	95.5	93.1	97.8
30–34	100.0	98.5	97.8	98.2	98.1		98.8	99.4	98.3
35–39		98.5	98.9	100.0	100.0		98.8	100.0	98.8
40–44		100.0	98.9				98.8		99.4
45–49			100.0				100.0		100.0

* < 2 mm = anergy.

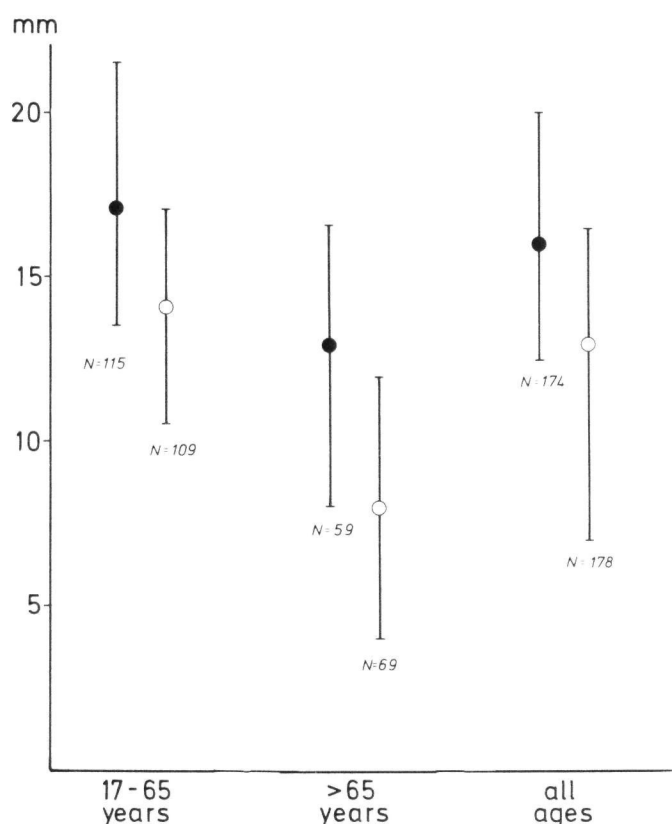


Fig. 1. Median "score" of seven antigens in 352 adult healthy volunteers. "Score" is the sum in mm of all median diameters of positive reactions. ● Males, ○ Females. ⊥ Interquartile range.

should be regarded as undesirable because of any hidden unknown side effects. The present *in vivo* technique involving recall-antigens is rapid, generally reproducible and does not require laboratory equipment (4, 9). Since it tests reactions to recall-antigens, the utility of the test is based on the use of relevant antigens, i.e. antigens in contact with the majority of a given population. In this context contact implies either exposure to the infecting micro-organism or vaccination with relevant antigens. In young children, isolated populations, and in others not being vaccinated, the test results will not reflect the general status of cell-mediated immunity in individuals. However, variations in exposure and vaccination status can partly be overcome by including a large spectrum of antigens in the test and by referring to local reference values. Division into age and sex groups seems relevant, primarily because of the differences in general immune function and because of probable differences in exposure to specific antigens in these groups.

The size and number of "positive" reactions vary with the test technique as well as the number, types, and concentrations of the antigens included. Even with the same equipment reactions may vary: Comparison of studies from different regions reveals quite large differences (3, 4, 7, 8, 10). This is also the conclusion of a large Swiss-French study of healthy adults under the age of 65 (13). Although good agreement between the French and Swiss populations was found with regard to the overall number of positive reactions, anergy frequency and score, significant differences between the two populations did exist concerning the number of positive reactions to tuberculin, tetanus and *Candida albicans*. The overall figures reported in the Swiss-French study and in a recent British study (10) are in agreement with those of the present study. North American children between 7 and 10 years also had scores and number of positive reactions at a rate similar to our study (3), whereas older children and adults in this area showed a higher number of positive reactions (7). Furthermore the adult study demonstrated higher scores with increasing age.

In Denmark during compulsory military service all males for several decades have been vaccinated against tetanus and tuberculosis if the Mantoux-reaction was negative. This could explain why in the male group reactions to these antigens are seen significantly more frequently and give rise to larger reactions to tuberculin. The age-dependency for reaction size seems to be common to all the antigens included. The difference between the 65 + years and the younger group is most pronounced for tetanus antigen, probably due to the lack of vaccination among older individuals. Although statistically significant, the incidence of reactions to diphtheria antigen is less reduced with age than observed for the other antigens. Vaccination with diphtheria toxoid was introduced as a routine to all children 35 years ago, but before that time a larger proportion had been exposed to the bacteria itself. This could have led to a stronger immune response.

Delayed hypersensitivity testing by intradermal injection of five antigens for the prediction

of postoperative infectious complications, has revealed conflicting results (1, 2, 12, 14). Using Meaking's criteria (11) Pietsch and co-workers (12) and Revhaug & Giercksky (14) found a significant correlation between an impaired response (anergy or relative anergy) and postoperative sepsis, whereas Ausobsky et al. (1) and Brown et al. (2) did not. The possible correlation between impaired cell-mediated immunity and postoperative sepsis has been assessed by the multipuncture system (Multi-test) in one investigation only by Dürig and co-workers (5) who found a significantly increased postoperative sepsis rate in anergic and relatively anergic patients.

Our results stress the importance of reference values stratified according to age and sex from local populations exposed to the same micro-organisms and vaccination programs.

REFERENCES

1. Ausobsky, J. R., Bean, P., Proctor, J. & Pollock, A. V.: Delayed hypersensitivity testing for the prediction of postoperative complications. *Br. J. Surg.* 69, 346-348, 1982.
2. Brown, R., Bancewicz, J., Hamid, J., et al.: Failure of delayed hypersensitivity skin testing to predict postoperative sepsis and mortality. *Br. Med. J.* 284, 851-853, 1982.
3. Corriel, R. N., Kniker, W. T., McBryde, J. L. & Lesourd, B. M.: Cell-mediated immunity in school-children assessed by multitest skin testing. *Am. J. Dis. Child.* 139, 141-146, 1985.
4. Delbrück, H., Schwarze, G., Scharding, B., et al.: Cutaneous testing with recall antigens in tumour patients and healthy subjects. Experiences with a new multitest system. *Tumor Diagnostik* 3, 18-24, 1982.
5. Dürig, M., Heberer, M. & Harder, F.: Technik und Bedeutung des Intracutantestes mit Recall-Antigenen in der Allgemeinchirurgie. *Chirurg* 53, 427-430, 1982.
6. Kniker, W. T., Anderson, C. T. & Roumiantzeff, M.: The multi-test system: A standardized approach to evaluation of delayed hypersensitivity and cell-mediated immunity. *Ann. Allergy* 43, 73-79, 1979.
7. Kniker, W. T., Anderson, C. T., McBryde, J. L., Roumiantzeff, M. & Lesourd, B.: Multitest CMI for standardized measurement of delayed cutaneous hypersensitivity and cell-mediated immunity. Normal values and proposed scoring system for healthy adults in the U.S.A. *Ann. Allergy* 52, 75-82, 1984.
8. Lesourd, B., Roumiantzeff, M., Wisniack, J., Biron, G., Loeper, J. & Moulias, R.: Un test simple d'immunité cellulaire au lit du malade: "la multipuncture". Définition d'une population française de référence. *Nouv. Presse Med.* 9, 3435-3442, 1980.
9. Lesourd, B. & Winters, W. D.: Specific immune responses to skin test antigens following repeated multiple antigen skin tests in normal individuals. *Clin. Exp. Immunol.* 50, 635-643, 1982.
10. Maxwell, A. P. & McCluskey, D. R.: Assessment of cell-mediated immunity in a British population using multiple skin test antigens. *Clin. Allergy* 16, 365-369, 1986.
11. Meakins, J. L., Pietsch, J. B., Bubenick, O., et al.: Delayed hypersensitivity: Indicator of acquired failure of host defence in sepsis and trauma. *Ann. Surg.* 186, 241-250, 1977.
12. Pietsch, J. B., Meakins, J. L. & MacLean, L. D.: The delayed hypersensitivity response: Application in clinical surgery. *Surgery* 82, 349-355, 1977.
13. Poirrot, P., Defayolle, M., Biron, G. & Roumiantzeff, M.: Measurement of delayed cutaneous hypersensitivity by the multitest system. *Schweizerisch-Deutscher Rheumatologenkongress*, 5-9 Oktober 1982, Basel.
14. Revhaug, A. & Giercksky, K-E.: Preoperative cutaneous hypersensitivity reaction related to postoperative complications following elective surgery. *Acta Chir. Scand.* 150, 279-283, 1984.
15. Roumiantzeff, M., Anderson, C., Beranger, G., Kniker, W. T. & Musetescu, M.: Antigens measuring cell-mediated immunity: Testing in experimental animals. In WHO-Center, (ed.): *Proceedings of the International Symposium on Experimental Animals and In Vitro Systems in Medical Microbiology*, Munich, Germany, 1976. Munich: WHO Collaborating Center, 174-202, 1977.

Address:

F. Moesgaard, M.D.
Stoltenbergsgade 1
DK-1576 Copenhagen
Denmark

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.