New Trends in Allergy II

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With a Foreword by O. Braun-Falco

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Contact-Allergy Time

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Abstract

The most commonly used techniques for the in vivo evaluation of the cellular immune response include intracutaneous testing with microbial recall antigens or sensitization with neoantigens. The reliability of these tests for the individual patient usually is low due to the lack of standardization and quantification. Moreover only the efferent branch of the immune response can be judged.

The dinitrochlorobenzene-contact allergy time (DNCB-CAT) is a quantitative approach for the assessment of the cellular immune response. 2% DNCB-ointment is applied on the upper arm in a 1 cm^2 area. On the following days patch-testing with 0.05% DNCB-ointment is done on the homolateral forearm in alternating localizations till an allergic contact dermatitis reaction appears.

As assessed in patients with malignant melanoma (MM, $n = 115$) and with lymphoproliferative disorders (LD, $n = 25$), the DNCB-CAT correlates with the age of the patients and can be expressed by a formula given by the age (years) × factor (MM = 0.16; LD = 0.17) + constant figure (MM = 5.5; LD = 4.3). There was no significant difference between the two groups or subgroups investigated.

By DNCB-CAT quantitative analysis of the cellular immune response in vivo is possible. It is an appropriate model for further investigations of the cellular immunity under different clinical, histological, prognostic, and therapeutic aspects.

Introduction

Immune status is an ill defined term, reflecting the capacity of the organism to exhibit immunologic defense mechanisms following antigenic stimulation. Evaluation of the immune status may be of interest in various disciplines, such as oncology, hematology, and transplantation medicine. In dermatology alteration of the immune status has been described in sarcoidosis, leprosy, atopic eczema, collagenosis, chronic infectious diseases, AIDS, malignant neoplasms

(including squamous and basal cell carcinoma, malignant melanoma and lymphoma), and several other conditions [1, 5].

Reports in the literature are controversial. For example in malignant melanoma, cell-mediated immunity assessed by recall antigens or by neoantigens has been claimed to be normal [22], impaired [19], and even increased [20]. The reasons for the contradictory results are manifold. They may be due to the method applied, to the patient and his disease, or to the test material or antigens used: Firstly the various in vitro and in vivo tests for the assessment of immune status can scarcely be compared since they reflect different functional capacities of the immune system. Secondly the contradictory results may be related to the patient and his disease in that factors claimed to modulate the immune response, like atopy [15, 16], clinical stage of tumoral diseases [14], sex [11, 17], or surgical stress [3], have been neglected in many of the reports on the immune status of patients tested. Thirdly the in vivo methods used are not standardized with respect to either the test procedure and the number and type of antigens tested or the evaluation of the test reaction [12]. Comparison of six recall antigens provided by two different manufacturers tested simultaneously in each of 20 patients revealed different test reactions in 10% for tuberculin, in 20% for trichophytin, in 30% for diphtheria, in 35% for streptococci or candidin, and in more than 50% for tetanus antigen (Table 1). In the same group of patients there was complete anergy in one patient tested with seven recall antigens from manufacturer M and in another patient tested with antigens from manufacturer B. The mean numbers of positive reactions in the two groups were 40% and 25% respectively (Table 2).

One step towards standardization and quantification of delayed type hypersensitivity tests for the evaluation of immune status is the multitest. The application of seven recall antigens and the control is standardized; the result is

Table 1. Comparison of six recall antigens from two different manufacturers tested in each of 20 patients

Recall antigen	% discordance
Tuberculin	10%
Trichophytin	20%
Diphtheria	30%
Streptococci	35%
Candidin	35%
Tetanus	55%

Table 2. Comparison of DTH reactions of recall antigens from two different manufacturers (M and B) ($n = 20$)

Manufacturer	M	B
Number of recall antigens	7	11
Complete anergy	5% (1)	5% (1)
Mean number of positive reactions	40% (3/7)	25% (3/11)

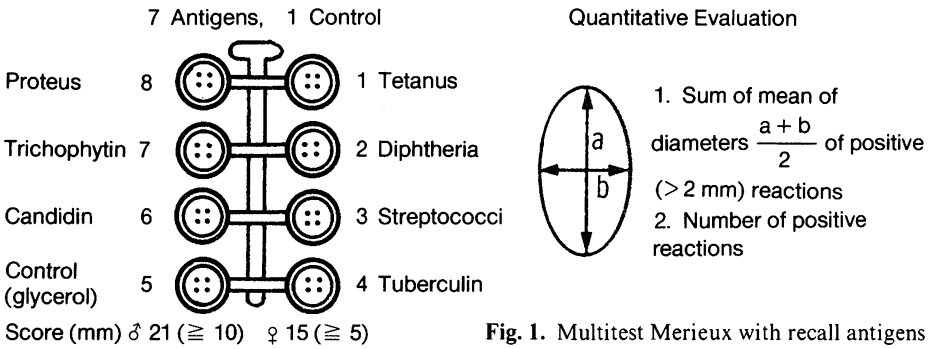

Fig. 1. Multitest Merieux with recall antigens

given in a score calculated from the number and the sum of diameters of positive test reactions (Fig. 1). The disadvantage, as with all tests using recall antigens, is that the multitest depends partly on regional vaccination programs, on geographic and epidemic factors, and on the sex of the patient without correlation to age [13]. Therefore testing with neoantigens like DNCB would seem more reliable [7].

Potential hazards of therapeutic use of dinitrochlorobenzene (DNCB), like mutagenicity or cross-reaction with other substances, can be ignored when it is used as a test allergen [2, 6, 8, 21]. The common procedure for DNCB testing consists in the application of a toxic 2% concentration of DNCB on the arm and a threshold challenge test with DNCB in various concentrations 2–4 weeks later. This neglects two important factors:

1. Only some of the patients will be sensitized at the time of the challenge test.
2. If the challenge test is performed when more time has elapsed since the first contact, the degree of sensitization will be slowly fading, so that determination of the threshold dose will not quantitatively reflect the immune status of the patient [10].

The disadvantage of all classical in vivo tests of the immune status – intracutaneous with recall antigens or epicutaneous with neoantigens – is the fact that only the efferent limb of the immune reaction process is measured, not the afferent limb, which is more complex, more important, and affords greater insight into the integrity or disturbance of the immune system [9].

Method and Patient Material

We tried to evaluate the complete process of development of hypersensitivity, as expressed by the time needed by the patient to build up an immunological reaction to DNCB, including both the afferent and the efferent limb (Fig. 2). A patch with 2% DNCB was applied on day 0 on a 1 cm^2 area of the upper arm. Subsequently 0.05% DNCB was applied daily on the forearm in alternating locations. The day when an allergic reaction appeared was registered. One week later a challenge test was performed in order to determine the threshold dose, using various concentrations of DNCB, from 0.001% to 0.05%.

Table 3. DNCB-CAT test results in 115 patients with malignant melanoma

		Patients		DNCB-CAT (days)		
		No.	Mean age	Mean	Shortest	Longest
Type	SSM	54	54	14	6	58
	NM	48	48	15	6	62
	LMM	3	59	23	10	49
	ALM	4	51	10	8	12
Stage	I	103	51	14	6	62
	II	8	47	14	9	25
	III	4	51	13	10	16
Level	II	10	56	14	8	30
	III	40	52	17	8	62
	IV	46	51	13	6	31
	V	5	51	12	10	14
Prognostic index (18)	0–1	29	51	14	8	62
	1.1–13	46	48	15	6	58
	>13	21	56	14	9	31

Abbreviations: SSM, superficial spreading melanoma; NM, nodular melanoma; LMM, lentigo maligna melanoma; ALM, acrolentiginous melanoma

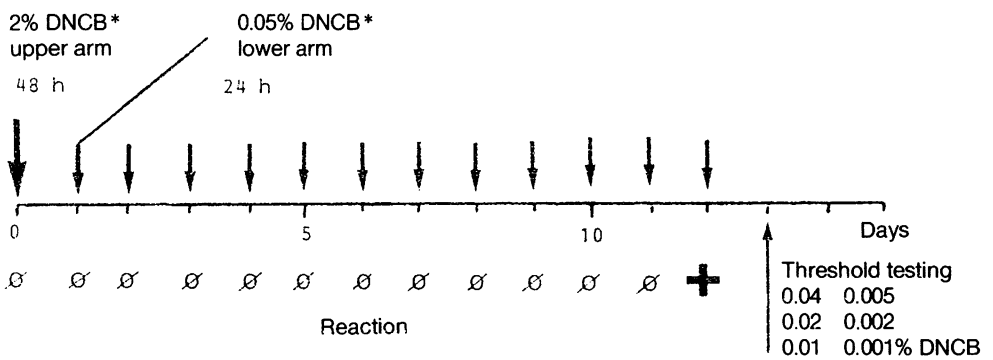

Fig. 2. DNCB-CAT for testing the immune status. * in Eucerin anhydricum

The test was applied to 115 patients with malignant melanoma (46 men, 69 women, age range 19–79 years, mean 51 years). Further information on their clinical features, stage, level, and prognostic index (PI [18]) is given in Table 3.

Results

The figures given in Table 3 illustrate the DNCB contact-allergy time (DNCB-CAT) in various subgroups of malignant melanoma patients. The shortest time measured (6 days) was found in one patient with superficial spreading melanoma (SSM) and in another with nodular melanoma (NM). Eleven patients

showed a DCNB-CAT of longer than 20 days; the longest, 62 days, occurred in a patient with stage I, level III, low risk (prognostic index below 1) NM.

No significant correlation could be detected between the DNCB-CAT in various clinical types of malignant melanoma (SSM, NM, lentigo maligna melanoma, acrolentiginous melanoma) and stage (I–III), level (II–V), or prognostic index score (< 1, 1.1–13, > 13).

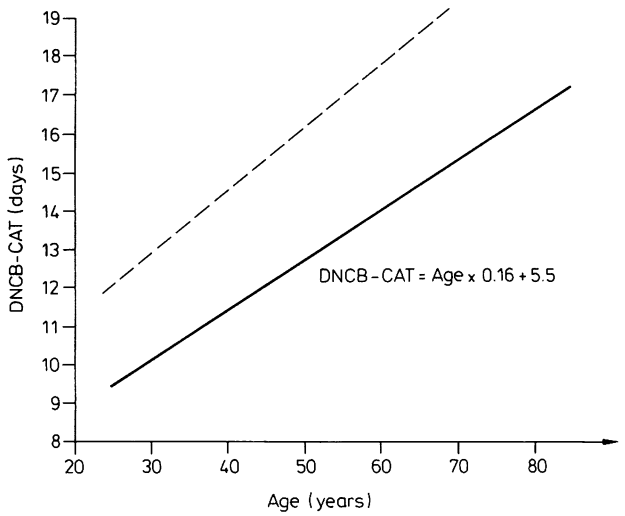

Fig. 3. Age-dependent regression curve of DNCB-CAT in malignant melanoma and 125% percentile (– –)

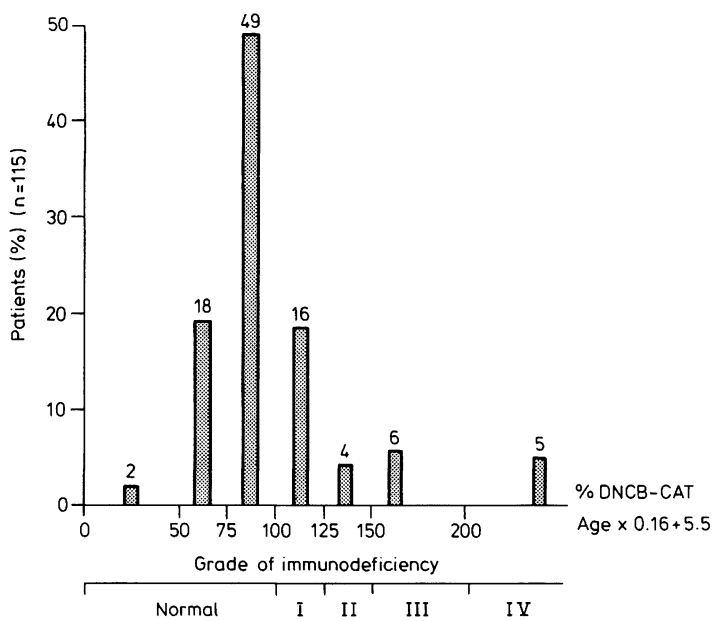

Fig. 4. DNCB-CAT exceeding the age-dependent calculated average: grades I–IV of immune deficiency

The most important finding was a strong correlation between DNCB-CAT and age of the patient. The expected DNCB-CAT was found to equal the age of the patient multiplied by a constant factor (0.16) plus a constant figure (5.5). Thus the DNCB-CAT in a 20-year-old patient is on average about half that expected in an 80-year-old patient (Fig. 3). Of the 115 patients, 69% ($n = 79$) exhibited a DNCB-CAT below the expected value as calculated by the above means. On the other hand 16% ($n = 18$) exceeded the expected figure by more than 25%, 11% by more than 50%, and 5% by more than 100% (Table 4, Fig. 4).

Table 4. Grading of immune deficiency by DNCB-CAT

Grade	% exceeding normal value (age × 0.16 + 5.5)	No. of patients	%
0 (normal)	0	79	69
I	≦ 25%	18	16
II	≦ 50%	5	4
III	≦ 100%	7	6
IV	> 100%	6	5

Table 5. Details of melanoma patients (n = 18) exhibiting an increased DNCB-CAT

Immuno-deficiency grade	% DNCB-CAT calculated	Age (years)	MM (type)	Stage[a]	Level	Depth of invasion (mm)	PI[b]	Follow-up (years)	Stage[c]
II	136	42	SSM	II	–	–	–	3.5	0
125%–150%	140	41	SSM	I	II	0.27	< 1	1	0
	140	41	SSM	I	II	0.85	3	3	0
	142	43	SSM	I	II	0.27	< 1	2	0
	146	45	SSM	II	IV	–	33	4.5	+
III	152	42	SSM	I	IV	1.1	5	2	0
151%–200%	152	74	NM	I	IV	1.1	5	3	+
	156	21	NM	II	V	–	–	0.2	+
	166	50	SSM	I	III	0.45	< 1	3	0
	168	46	NM	I	III	0.48	2	3.5	0
	177	52	NM	II	IV	5.1	82	1.5	0
	184	68	NM	I	IV	–	32	3	II/0
IV	203	56	SSM	I	II	0.68	< 1	2	0
> 200%	208	42	ALM	I	IV	3.3	3	2	+
	248	54	SSM	I	III	1.37	5	2.5	0
	295	67	LMM	I	III	1.2	13	4	0
	365	69	NM	I	III	0.43	< 1	3	0
	433	47	SSM	I	III	–	9	3	0

[a] At time of diagnosis
[b] Prognostic index [18]
[c] At end of follow-up

Table 6. DNCB-CAT and multitest results in 15 patients with malignant melanoma

DNCB-CAT \ Multitest	Normal	Pathologic
Normal	9 (60%)[a]	3 (20%)[b]
Pathologic	2 (13%)[b]	1 (7%)[a]

[a] Concordance between tests = 67%
[b] Discordance between tests = 33%

Table 7. Correlation between DNCB-CAT and DNCB threshold concentration in 13 melanoma patients

DNCB-CAT[a]	Degree of hypersensitivity	
	High[b]	Low[c]
+	2 (15%)[d]	8 (62%)[e]
−	1 (8%)[e]	2 (15%)[d]

[a] + = normal or below; − = > 25% above normal
[b] 0.005%, 0.002%, or 0.001% DNCB
[c] 0.04%, 0.02%, or 0.01% DNCB
[d] Concordance = 30%
[e] Discordance = 70%

If the patients whose DCNB-CATs were higher than expected were categorized into four immunodeficiency groups (I = 101% − 125% of normal age-related DNCB-CAT; II = 126% − 150%; III = 151% − 200%; IV > 200%) and the patients of groups II − IV were listed in order of their DNCB-CAT (Table 5), no correlation was seen either with the type of melanoma or with stage, level, prognostic indices, or survival within the different categories.

In 15 patients we compared the result of the Merieux multitest with the DNCB-CAT test. A multitest score below 5 in women and below 10 in men and a DNCB-CAT exceeding the expected value by more than 25% were considered pathologic. Concordance between the multitest and the DCNB-CAT test was found in about two-thirds of the patients tested (Table 6).

Correlation between the DNCB-CAT and the degree of sensitization expressed by the threshold concentration of DNCB was found in only about one-third of the 13 patients tested (Table 7).

Discussion

Abnormalities of the cell-mediated immune status have been reported in a variety of dermatoses, including inflammatory and proliferating disorders [5]. It has been speculated that tumor induction and tumor growth are controlled by

specific immune reactions against tumor cells, including enhancing and blocking mechanisms. However, there is little evidence that, for example, PHA stimulation of peripheral lymphocytes, the type and degree of the peritumoral infiltrate [4], or reactivity against recall antigens or neoantigens provide any reliable information as to the induction and growth of solid tumors. Nevertheless, in advanced stages of tumoral diseases with widespread metastases the immune system may be severely affected and a single test may elucidate dysfunctioning delayed type sensitivity provided the procedure is standardized and individual factors (e.g., age) are considered. Moreover it is important that the afferent and efferent limbs of the delayed type reactivity are measured. Therefore neoantigens are preferable to recall antigens.

The DNCB-CAT test is recommended as a standardized test for the assessment of immune status since it is simple, covers the complete process of building up delayed type hypersensitivity, and considers the most important individual factor, namely age. When using the DNCB-CAT test, grades (I–IV) of immunodeficiency can be differentiated in accordance with the percentage by which the actual DNCB-CAT exceeds the average-figure calculated for the individual age group.

Summary

The DNCB-CAT test is a quantitative approach for the assessment of the cellular immune response. 2% DNCB ointment is applied on the upper arm over a 1 cm^2 area. On subsequent days patch testing with 0.05% DNCB ointment is done on the homolateral forearm until an allergic contact dermatitis reaction appears. By this the afferent and the efferent limb of the delayed hypersensitivity reaction is measured.

In a group of 115 patients with solid tumors (malignant melanomas) DNCB-CAT correlated with the age of the patient and could be expressed by the following equation:

$$[\text{DNCB-CAT} = \text{Age (years)} \times 0.16] + 5.5$$

Deviations from the calculated agedependent regression curve were categorized into grades I–IV of immunodeficiency.

DNCB-CAT is an appropriate model for quantitative age-related analysis of cellular immune responsiveness in relation to various clinical, histologic, prognostic, and therapeutic factors.

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