Acta Derm Venereol (Stockh) 1985; 65: 385–389

Correlation between Quantitative in vivo and in vitro Responses in Nickel-Allergic Patients

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Al-Tawil NG, Berggren G, Emtestam L, Fransson J, Jernselius R, Marcusson JA. Correlation between quantitative in vivo and in vitro respones in nickel—allergic patients. Acta Derm Venereol (Stockh) 1985; 65: 385–389.

Forty-three nickel-allergic patients (40 females and 3 males) participated in this study which was designed to elucidate the correlation between quantitative in vivo and in vitro responses. All were patch-tested with various concentrations of nickel-sulphate serial-diluted in water. The lowest concentration eliciting a cutaneous reaction (indicating the severity of the allergy) was recorded for each individual and compared with the in vitro lymphocyte response, using the lymphocyte transformation test. The best correlation coefficients obtained were 0.42 and 0.46, tested for linear and logarithmic correlation respectively. However, there were individuals who showed a weak cutaneous response and high lymphocyte reactivity. The converse situation was also found, however, indicating that other mechanisms than those mediated via lymphocytes may be of significance in the patch test response. (Received January 10, 1985.)

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The incidence of contact allergy to nickel is high, the frequency in Europe being some 4–10%, in North America even higher (7). The allergy arises more often in females than in males (8) and the causes of nickel sensitivity are various. It can result from environmental and from occupational exposure of different kinds (9).

The diagnosis of nickel sensitivity is based on a meticulous anamnesis and patch testing. Despite the fact that the test procedure is thoroughly standardized, the results are difficult to interpret so as to distinguish between allergic and irritant reactions (5, 6). Immunohistological studies of the skin when challenged with irritant or allergic compounds have not revealed any qualitative difference, merely a quantitative difference in the cellular infiltrate in the two situations. Most lymphoid cells had markers of helper/inducer cells while only a few of cytotoxic and immunoglobulin bearing cells (17). On histological grounds one would therefore expect that the peripheral blood lymphocytes responding to in vitro exposure to the antigen would be T-helper cells.

In a previous study (1) we commented on our data regarding the dose response and kinetic conditions necessary for optimal in vitro response of peripheral blood lymphocytes from patients with similar allergy challenged with $NiSO_4$. We have shown that the lymphocyte response reflects the proliferation of T cells requiring antigen-presenting adherent cells. However, we have been unable to demonstrate the development of cytotoxic T cells tested against PHA-blasts (unpublished data).

In the present work we studied the correlation between the quantitative response in vivo and in vitro in nickel-sensitive patients.

MATERIAL AND METHODS

Patients

The 43 patients (40 females and 3 males) who participated were outptients at the Department of Dermatology, Karolinska Hospital, Stockholm, Sweden, who consulted the clinic for cutaneous disease. Their mean age was 42 years (range 18–82).

Patch testing

A standard patch test (10) using Finn Chambers was employed (15) and reading was done as followed: +=erythema, ++=erythema and papules, and +++=erythema, papules, vesicles. Those who proved positive for nickel (2+ or more) were selected for the study. In order to further evaluate the cutaneous response, a serial dilution test was conducted (18, 19) using $NiSO_4$ in water. The following concentrations were used: 2.5%, 1.25%, 0.625%, 0.312%, 0.156%, 0.078%, 0.039%, 0.019%, 0.009%. All tests were performed when the patients had been free of eczema for at least 2 weeks. Only 2+ reactions or more were considered in the two test situations and the response for the lowest concentration was noted (threshold of sensitivity) (18, 19).

Laboratory methods

The in vitro method used has been described before (1) and will be only briefly recapitulated. After purification of the lymphocytes the cells were washed and suspended in tissue culture medium. Thereafter they were distributed on round bottom micro-culture plates in triplicate (1). Each well contained 1×10^5 lymphocytes. $NiSO_4$ was added, to give final concentrations of 40.39, 80.77 and 161.54 μmol/l corresponding to 6.25, 12.5 and 25 μg/ml respectively. The cultures were incubated at 37°C in humidified air containing 5% CO_2. To each well, 1 μCi of methyl ($_3$H) thymidine (Amersham International plc, Amersham, UK) spec. activity 5 Ci/mmol) was added 24 hours before harvesting on days 5 and 6. The filters were then dried and the uptake of isotope was measured in a liquid scintillation counter (LKB, Wallac, 1216, Rackbeta II, Finland).

Lymphocytes from non-allergic healthy subjects were always included as negative controls and treated in the same way as those of the sensitive patients. All cells were tested against PHA (final concentration 50 μg/ml) as a positive control. The experiments were performed with cells from 3–5 patients and at least one healthy control at a time.

Results were expressed as increment counts per minute (icpm) where the mean count per minute (cpm) of triplicates of unstimulated cultures was subtracted from the mean cpm of the cultures stimulated with $NiSO_4$ and as stimulation indices (SI), where the mean cpm of the stimulated cultures was divided by the mean cpm of unstimulated cultures. Correlation coefficients for logarithmic and linear correlation between the strength of the in vivo and in vitro reactivity were calculated for each in vitro concentration of nickel sulphate used on each day.

RESULTS

All patients fulfilled the criteria for in vitro nickel allergy (1). The correlation coefficients are listed in Table I. Because of the technical failure of some cultures the number of patients included varied between 37 and 43. It can be seen that the coefficients are generally lower when tested for icpm than when tested for SI. The best coefficients for both linear and logarithmic correlation were 0.42 and 0.46 respectively when the lymphocytes were tested with 80.77 μmol/l $NiSO_4$/ml and harvested on day 5. Thus there is a low correlation between the strength of the in vitro lymphocyte response and in vivo total cutaneous reactivity.

However, Fig. 1 illustrates the spread of individual values. Thus there are subjects who react positively at the lowest concentration of $NiSO_4$ but with low in vitro reactivity. The converse situation is also illustrated. The pattern is similar for each concentration of $NiSO_4$ harvested on days 5 and 6 (data not shown). The lymphocytes of the controls responded in all concentrations in a pattern similar to that of the figure and as previously described (1).

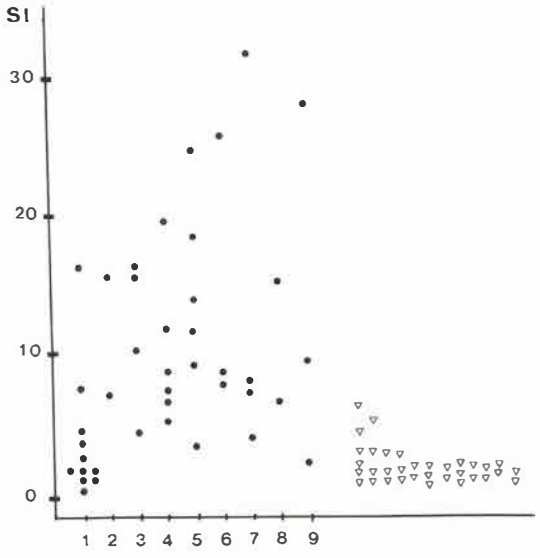

Fig. 1. Correlation between the strength of the in vitro and in vivo response. Ordinate: Stimulation indices (SI). Abscissa; Degree of cutaneous reactivity: 1, a positive patch test response using 2.5% nickel sulphate; 2, 1.25% and so on (see materials and methods). The figure illustrates the lymphocyte response on day 5 using 80.77 μmol/l. The response of the control lymphocytes are visualized with ▽.

Table I. *Correlation between the quantitative in vivo and in vitro response*

Coefficients for logarithmic and linear correlations are given. The calculations have been made on both increment counts per minute and stimulation indices on days 5 and 6 of culture

	Stimulation index	Increment cpm
40,39 μmol/l		
Day 5, n=37		
Linear c[a]	0.36	0.20
Logarithmic c	0.41	0.28
Day 6, n=39		
Linear c	0.27	0.22
Logarithmic c	0.43	0.39
80,77 μmol/l		
Day 5, n=41		
Linear c	0.42	0.29
Logarithmic c	0.46	0.42
Day 6, n=43		
Linear c	0.22	0.20
Logarithmic c	0.33	0.34
161,54 μmol/l		
Day 5, n=41		
Linear c	0.40	0.31
Logarithmic c	0.43	0.42
Day 6, n=43		
Linear c	0.19	0.27
Logarithmic c	0.27	0.37

[a] c=correlation.

DISCUSSION

To estimate the severity of the nickel allergy and to investigate whether a response is irritant or allergic in vivo, the serial dilution test is performed (18, 19). Reactions to an allergen gradually diminish in severity the weaker the solution is; whereas reactions to an irritant cease abruptly below a certain concentration (18, 19). The lowest concentration of the compound that elicits a cutaneous response constitutes the threshold of sensitivity, i.e. reveals the intensity of the allergy. In this study we have investigated the correlation between the strength of the in vivo sensitivity, and that of the in vitro reactivity. The results showed a weak correlation (Table I). Moreover, there were individuals who showed a weak response in vivo yet a high lymphocyte reactivity in vitro, and vice versa (Fig. 1). The reasons for this can only be speculated upon.

One of the reasons for the apparent contradiction could be the selection of patients. They came to the Dermatology Clinic because of a cutaneous disease and the patch test was performed as a part of the examination. Although we conducted the tests when the patients had been free of eczema for a while we cannot rule out an unspecific individual reactivity of the skin to $NiSO_4$, which might be related to the skin disease itself, rather than to a suspected allergy at lymphocyte level.

Mitchel (14) has described the phenomenon of "angry back" syndrome. These cutaneous responses would occur for some substances applied close to a strongly positive allergic patch-test site or adjacent to an area of active eczema. This could also happen, when eczema was present at a distance from the test site. A recently healed eczema would also have the same effect.

Another possibility might be individual factors, among which the penetration of the test substance through the stratum corneum and epidermis could be relevant. For the study we used $NiSO_4$ diluted in water—the procedure in current use at our Department. Studies have previously shown that the highest yield of positive patch-test results and optimal penetration through the skin layers is obtained when using water rather than petrolatum as diluent (19). We therefore consider that the skin of our patients was challenged optimally. The problem of percutaneous penetration could be circumvented by employing the intracutaneous test (12). However, since we have shown that the in vivo patch test in itself might trigger the specifically sensitized lymphocytes (unpublished observation), we hesitate to perform such a study.

In this paper we have considered only the proliferative response of lymphocytes and the total cutaneous response, but we are aware that other cells are also involved. Askenase et al. (13) have shown that the elicitation of the delayed type of hypersensitivity in mice requires the T cell-dependent mast-cell release of vasoactive amines. Furthermore, cultured normal human epidermal cells have been shown to produce a thymocyte activating factor (8, 13). A thymopoietin-like substance has also been found mainly in the basal layer of keratinocytes in the epidermis (12). These latter findings indicate that other mechanisms in addition to those mediated via specifically sensitized T cells could lead to a positive eczematous patch test response.

Besides the above mentioned factors, the patch-test technique itself possesses pitfalls (5). To circumvent some of these one would like to study the quantitative response in vivo and in vitro in *healthy* individuals (as regards the skin) with a positive patch-test reaction and positive lymphocyte transformation test for the substance in question.

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