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IN VITRO LYMPHOCYTE TRANSFORMATION TO NICKEL: A STUDY OF NICKEL-SENSITIVE PATIENTS BEFORE AND AFTER EPICUTANEOUS AND ORAL CHALLENGE WITH NICKEL

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Abstract. An in vitro lymphocyte transformation test (LTT) was performed in 8 patients with nickel contact dermatitis and in 4 control persons. The mitogenic response was of the same magnitude before patch testing, after patch testing and after oral challenge with nickel. When purified protein derivative (PPD) was used as the antigen, there was no difference between the LTT response of patients and controls before and after patch testing. After oral challenge with nickel, however, the patients' responses to PPD were significantly increased when compared with the responses after patch testing. The responses of the controls were unchanged. The LTT levels of the patients were approximately the same before and after patch testing, but were markedly increased after oral challenge.

Key words: Nickel allergy; Lymphocyte transformation; Epicutaneous challenge; Oral challenge

A group of nickel-sensitive persons can be distinguished from a group of controls by the lymphocyte transformation test (LTT) using nickel sulphate as the antigen (2, 3, 5, 9). When the individual nickel-sensitive patient is tested, however, the transformation level may differ only slightly from that of a control person (2, 9). The LTT cannot, therefore, be used to identify all patients who have a positive nickel patch test. It is not known whether the LTT can be used to detect nickel sensitivity in persons who are suspected to be nickel sensitive yet who have negative nickel patch tests.

The level of transformation increases following epicutaneous challenge in humans with experimentally induced allergic contact dermatitis (8). The dermatitis of some nickel-sensitive persons flares up following epicutaneous and/or oral challenge (1, 4). The urinary excretion of nickel has been used as an indicator for the absorption of orally ingested nickel (6).

The present study was designed to show whether the results of the LTT are altered following patch testing and oral challenge with nickel sulphate when nickel sulphate is used as the antigen.

MATERIAL AND METHODS

Eight patients with eczema compatible with nickel contact dermatitis participated in the investigation. All the patients had been patch tested with the European Standard Series including 5% nickel sulphate in petrolatum within the preceding year. The patch tests were read after 48 and 72 hours, and the nickel tests were positive, i.e. showed erythema, infiltration, papules and/or vesicles for all the patients. Three patients also had weakly positive patch tests to cobalt (1% cobalt chloride in petrolatum).

Four persons with no history of dermatitis served as controls. In order to avoid stimulation of the lymphocytes by nickel, neither patients nor controls were patch tested immediately prior to the first LTT. Neither patients nor controls had any recent history or symptoms of infectious disease. Leukocyte and differential counts were made prior to and at the conclusion of the investigation.

The tests listed in Table 1 were carried out for both patients and controls. Urine sampels (the first voiding of the day) were collected from both groups. The nickel content in urine was determined by flameless atomic absorption (Perkin-Elmer 305B, with HGA-70 background corrector) following acid digestion and extraction into

Table 1. *Timetable for the tests performed*

Day	Test
0	Urinary excretion of nickel
1	Lymphocyte transformation test (LTT); Nickel patch test
3	Urinary excretion of nickel
4	Patch test read
15	LTT
22	Oral challenge with 4 mg nickel; Urinary excretion of nickel
23	Urinary excretion of nickel
24	Urinary excretion of nickel
25	Urinary excretion of nickel; Evaluation of clinical condition
36	LTT

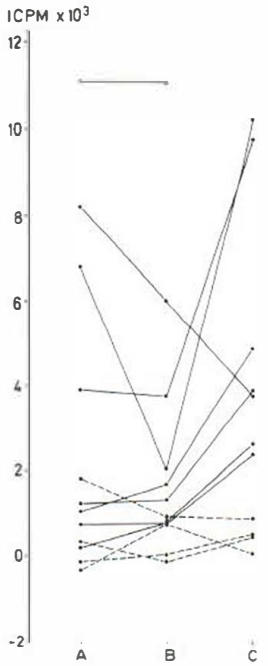

Fig. 1. Results of lymphocyte transformation for 8 patients —— and 4 controls – – – before patch testing (A), after patch testing (B) and after oral (C) using a nickel dilution of 1 : 100 as the antigen and a culture period of 144 hours. The results are expressed as increment counts per minute; for explanation see text. One patient was not challenged orally.

methylisobutylketone as a pyrrolidinedithiocarbamate complex (7).

The LTT was performed as previously described (9). Nickel sulphate $NiSo_4 7H_2O$, Merck 6725) was used as the antigen (hapten). From a stock solution of 1 w/v% nickel sulphate in saline, dilutions of 1 : 100 and 1 : 200 were made with RPMI-1640 medium (Gibco). The concentrations in the culture tubes were 3.6×10^{-5} M and 7.2×10^{-5} M, respectively.

The general cellular immune response of the patients and the mitogenic activity of the lymphocytes were tested using Phytohemagglutinin (PHA, Difco), 400 μg/ml, pokeweed mitogen (PWM, Gibco), diluted 1 : 500 and Concanavalin A (ConA, Pharmacia) 500 μg/ml as mitogens. Purified protein derivative (PPD, Statens Seruminstitut, Copenhagen) 10 μg protein/ml and a *Candida albicans* extract (0.3 μg protein/ml) were used as recall antigens.

The cultures were made in round-bottomed culture tubes, each containing 10^5 lymphocytes, 50 μl mitogenic solution or antigen and 0.5 ml RPMI-1640 medium. The following reagents were added to each litre of medium: 480 000 units of penicillin, 0.5 g streptomycin, 50 mM hepes, 1.2 mmol glutamine and 20 000 units of heparin. All cultures were made in triplicate. 24 hours before harvesting with a semi-automatic harvester (Skatron) 0.05 μCi ^{14}C-labelled thymidine (NEN Chemicals) was added to each culture tube. The radioactivity was determined by liquid scintillation counting. In the following, the results are expressed as increment counts per minute (ICPM), i.e. counts per minute (CPM) of stimulated culture (median of triplicates) minus CPM of unstimulated cultures (median of triplicates).

Culturing took place at 37°C in humidified air to which 5% CO_2 was added. The culture period was 96 hours when mitogens were added to the culture tubes, 120 hours when recall antigens were added, and 120 and 144 hours for those tubes to which nickel sulphate was added.

The nickel patch test using 5% nickel sulphate in petrolatum and Pirilä's aluminium discs sealed with tape were applied to all the patients and controls on day 1. The tests were read on day 4.

A nickel tablet containing 4 mg nickel as nickel sulphate in lactose was given on day 22. The clinical evaluation after ingestion of the tablet was made on day 25 and consisted of a combined evaluation of such symptoms as itching and physical findings such as the number of vesicles and the presence of edema and erythema.

RESULTS

All 8 patients had positive nickel patch tests with erythema, infiltration and papules, as well as, in some cases, edema and vesicles. One patient with eczema on both hands experienced a flare following the patch test. The dermatitis spread to the arms and legs and to an area surrounding the patch test site. This patient was not challenged orally. She had the highest level of lymphocyte transformation recorded both before and after patch testing (Fig. 1).

None of the control persons had positive nickel patch tests.

The dermatitis flared up in 4 of the 7 patients who ingested the nickel tablet. The flare occurred 1 to 2 days after ingestion, peaked after approximately 3 days, and subsided after 7 to 10 days. In one of these patients, a widespread erythema was seen one hour after ingestion of the nickel tablet. 1 to 2 days later, a moderate aggravation of this patient's hand dermatitis was seen. The erythema subsided after 2 days. One patient whose dermatitis was aggravated noted eruption of eczema in areas which had been involved 10 years earlier, but which had remained clear since then.

The activity of the dermatitis did not vary significantly at any time other than following ingestion of the nickel tablet.

The patients and the controls had a uniform response to the mitogens. There were no differences in the responses to mitogens prior to patch testing,

Table II. *Results of lymphocyte transformation in 8 nickel-sensitive patients and 4 controls using the mitogens phytohemagglutinin (PHA), pokeweed mitogen (PWM), and Concanavalin A (ConA)*

The results are expressed as the range and median (in parentheses) of increment counts per minute; for explanation see text

	PHA	PWM	ConA
Before patch testing	26 030–56 671 (43 745)	5 670–20 056 (12 659)	12 492–35 443 (22 926)
After patch testing	23 459–64 792 (35 235)	5 950–24 330 (14 190)	13 291–50 416 (20 291)
After oral challenge	28 826–51 567 (40 716)	8 520–27 738 (14 218)	14 891–35 667 (22 194)

following patch testing, or following oral challenge (Table II).

There was no statistically significant difference between the responses of patients and controls either before or after patch testing or after oral challenge with nickel when the antigen PPD was used ($p>0.05$, Mann-Whitney test) (Table III). In the patient group, however, there was a significantly increased transformation following oral challenge compared with the level after patch testing ($p<0.05$, Wilcoxon test). There was no similar increase among the controls.

Prior to patch testing, the results of the LTT using nickel sulphate as the antigen showed a significant difference between patients and controls except when the 1 : 100 dilution of the nickel antigen was used for a culture period of 144 hours (Table IV). There was some overlap between the two groups following a culture period of 144 hours. When the test was repeated after patch testing, no definite changes in the responses of either patients or controls could be seen. Following oral challenge, however, the median level of transformation in the patient group showed an increase compared with the levels before and after patch testing. The one exception to this was in the results following a culture period of 120 hours with a 1 : 100 dilution of the nickel antigen (Table IV). The increase was in no instance statistically significant at the 0.05 level (Wilcoxon test).

No increase in the transformation levels of the controls was seen following the oral challenge.

Details of the results using a nickel dilution of 1 : 100 and a culture period of 144 hours are presented in Fig. 1. The LTT results which provided the best distinction between patients and controls were obtained after oral challenge (Table IV).

There was no difference in transformation levels among patients whose dermatitis flared after oral challenge and those whose dermatitis remained unchanged. However, there were only a few patients in each group. The patient whose LTT response decreased throughout the period of investigation experienced aggravation of the dermatitis following oral challenge.

In Table V the urinary excretion of nickel following oral challenge with nickel sulphate is recorded for 7 patients and 4 controls. One patient failed to collect urine sampels after the challenge. There was no correlation between the severity of dermatitis and nickel excretion at the outset of the study. No marked deviations in nickel excretion were seen when the results prior to and after patch testing were compared. Increased excretion was seen, however, on the first or second day after ingestion of the nickel tablet for both patients and controls.

Table III. *Results of lymphocyte transformation in 8 nickel-sensitive patients and 4 controls using purified protein derivative (PPD) as the antigen*

The results are expressed as the range and median (in parentheses) of increment counts per minute; for explanation see text

	Patients	Controls
Before patch testing	3 854–11 889 (6 292)	846–18 387 (13 918)
After patch testing	3 525– 9 990 (6 307)	2 529–22 102 (11 248)
After oral challenge	4 607–11 597 (10 180)	880–18 194 (10 955)

Table IV. *Results of the lymphocyte transformation test before patch testing, after patch testing, and after oral challenge using 1 : 100 and 1 : 200 dilutions of the nickel antigen and culture periods of 120 and 144 hours*

The results are expressed as range and median (in parentheses) of increment counts per minute; for explanation see text. The Mann-Whitney test was used for the statistical analysis

	120 hours		144 hours	
	1 : 100	1 : 200	1 : 100	1 : 200
Before patch testing				
Patients	619–5 862 (2 079) $p<0.01$	244–6 677 (1 521) $p<0.01$	191–11 186 (2 548) $0.05<p<0.10$	1 140–11 613 (2 671) $0.01<p<0.05$
Controls	−122–496 (350)	−497–95 (−94)	−331–1 826 (123)	−167–1 386 (191)
After patch testing				
Patients	250–6 203 (2 313) $0.01<p<0.05$	360–6 543 (1 430) $0.01<p<0.05$	800–11 106 (1 867) $0.01<p<0.05$	390–12 460 (1 833) $0.05<p<0.10$
Controls	−64–1 043 (32)	−85–869 (43)	−112–940 (382)	−43–741 (525)
After oral challenge				
Patients	555–8 543 (2 185) $p<0.01$	371–6 148 (1 996) $p<0.01$	2 388–10 204 (3 867) $p<0.01$	365–6 042 (4 120) $0.01<p<0.05$
Controls	48–384 (157)	−15–168 (37)	56–498 (477)	7–1 459 (332)

For 2 patients (K. H. and B. L.) only a moderate increase in nickel excretion was seen. Neither of these patients experienced flare of the dermatitis after oral challenge, but for both of them the LTT response increased. The patient with a decreased LTT response after oral challenge (H. H.) had a high level of nickel excretion after challenge.

DISCUSSION

The LTT results for nickel-sensitive patients appear to be most distinct from those obtained for controls when the test is performed after an oral challenge with nickel. Patch testing prior to the LTT did not produce increased levels of transformation, contrary to the observations made when persons with experimentally induced contact dermatitis caused by dinitrochlorobenzene (DNCB) were challenged with DNCB (8).

The 2 week interval between challenge and LTT was chosen on the basis of the results obtained after epicutaneous challenge in humans with experimentally induced contact dermatitis (8). The interval between epicutaneous challenge and LTT following

Table V. *Urinary excretion of nickel for 7 patients and 4 controls (μg nickel per 100 ml urine)*

Patients' initials	Before patch tests	After patch tests	After oral challenge			
			Day 1	Day 2	Day 3	Day 4
K. H.	0.7	0.4	1.2	1.1	1.3	0.9
S. W.	1.5	1.3	2.0	6.4	4.0	4.1
G. K.	0.9	0.9	5.4	2.3	2.0	1.7
K. P.	0.2	0.7	0.4	–	–	–
H. H.	0.1	0.1	8.0	3.6	2.4	1.9
G. H.	0.1	0.1	0.1	2.8	1.2	1.0
B. L.	0.5	1.8	0.8	1.4	2.0	0.1
Mean	0.6	0.8	2.6	2.9	2.2	1.6
Controls' initials						
N. V.	0.7	0.8	3.9	2.4	1.1	1.2
K. V.	0.8	1.1	6.5	2.9	3.1	2.1
H. M.	0.3	2.8	0.3	7.2	6.7	2.0
T. M.	2.8	4.5	12.3	11.8	4.4	2.8
Mean	1.2	2.3	5.8	6.1	3.8	2.0

oral challenge was 35 days (Table I). Although this interval reduces the likelihood of an additive effect of epicutaneous and oral challenge, such an effect can be excluded only by duplicating the experiment with two groups of patients—one to be challenged epicutaneously, the other orally. The likelihood of an additive effect is decreased further as no increase in LTT response was seen after patch testing.

The increased level of transformation after oral challenge was seen both in patients who experienced clinical aggravation and those who did not. This would indicate that both clinical evaluation and LTT are useful in determining the significance of the oral ingestion of nickel in maintaining nickel dermatitis. The increase in nickel excretion after ingestion of the nickel tablet indicates that all patients and controls absorbed nickel from the gastrointestinal tract.

The LTT in this study was for practical reasons performed with cells from the peripheral blood. Thus we did not study the cells actually producing damage in the skin but rather an "excess" of memory cells present in the peripheral blood. We observed an increase in LTT response to nickel after oral challenge but not after patch testing, indicating that oral challenge is a more effective booster.

Svejgaard et al. (9) noted that individual controls without symptomatic nickel allergy respond significantly to nickel in the LTT. This type of reactivity, which was also seen in one of the controls (Fig. 1) in the present study, may be due to non-specific mitogenic properties of nickel or to latent nickel allergy in the controls in question. None of the controls in the current study showed significantly increased responses to nickel after patch testing or oral challenge; it is therefore unlikely that any of them have latent allergy.

It is also concluded that ingestion of nickel may be an important factor in the maintenance of nickel dermatitis, since oral challenge with nickel sulphate was followed by 1) absorption of the metal salt; 2) an increase in the transformation level of lymphocytes; and 3) flare of the dermatitis in some of the patients tested.

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