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LYMPHOCYTE TRANSFORMATION INDUCED BY NICKEL SULPHATE: AN IN VITRO STUDY OF SUBJECTS WITH AND WITHOUT A POSITIVE NICKEL PATCH TEST

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Abstract. Lymphocytes from 8 patients with contact dermatitis and a positive nickel patch test, 7 patients with contact dermatitis due to other factors and with a negative nickel patch test, and 9 other subjects, 7 of whom suffered from other dermatological disorders, were tested with the lymphocyte transformation test (LTT), using nickel sulphate in various concentrations. All except one of the nickel allergics showed a significant response to nickel, whereas the controls showed borderline (3 patients) or no response (12 patients). These observations confirm previous findings indicating that nickel hypersensitivity can be diagnosed in vitro. This may be of importance in cases of acute contact dermatitis where patch testing is undesirable. Some individuals with a negative nickel patch test responded significantly though weakly to nickel in the LTT, indicating that apart from acting as a specific antigen (hapten?) nickel sulphate may also have weak non-specific mitogenic properties.

Key words: Lymphocyte transformation; Nickel sensitivity; In vitro test; Contact dermatitis

The diagnosis of contact dermatitis is usually established by patch testing. However, when applied in the acute phase, patch testing with the specific antigen may give rise to severe exacerbation of the dermatitis. Moreover, false-positive (non-specific) patch tests can also occur in such cases (2). Accordingly, the development of specific in vitro tests would be of great advantage.

Nickel is one of the most frequent causes of allergic contact dermatitis (2). Early attempts to diagnose nickel hypersensitivity in vitro by means of the lymphocyte transformation test (LTT) failed, however, because nickel stimulated lymphocytes from sensitive as well as non-sensitive persons (1, 8). Recently several groups have provided evidence indicating that this test may be used to distinguish between nickel-allergic patients and controls (3, 5, 6, 7). Most recently, Gimenez-Camarosa et al. (4) were able to distinguish between two groups of nickel-sensitive patients, one responding only to low and one responding mainly to high concentrations of nickel sulphate.

The purpose of the present investigation was to determine whether our LTT assay, which uses only 10^5 mononuclear cells per test tube, could be used to diagnose nickel hypersensitivity. In addition, to further corroborate the specificity of the test, we investigated a number of patients suffering from contact dermatitis caused by other allergens. Studies on such controls have not been reported previously.

SUBJECTS

Three groups of individuals were included in the study (Table I):

(i) Eight patients with acute or subacute dermatitis compatible with a contact dermatitis due to nickel and a positive patch test to nickel. Patch testing was carried out with the European standard series and read after 48 and 72 hours. Nickel was applied as 5% nickel sulphate in petrolatum. Infiltration and papules or vesicles were required in order to describe a test as positive. Two of the patients were tested more than 6 months before LTT, 2 were tested more than 3 months before and 4 immediately preceding the LTT. In 2 of the patients positive reactions to other contact allergens were found in addition.

(ii) Seven patients with acute or subacute contact dermatitis with a negative patch test to nickel. Four of these patients had positive patch tests to other allergens. The intervals between patch testing and LTT are shown in Table I.

(iii) Nine persons without contact dermatitis. Seven of these individuals suffered from other dermatological disorders (Table I) while 2 had no history of skin disease. These 9 individuals were patch tested after the LTT.

Table I *a, b, c. Review of subjects, results of patch testing, clinical manifestations and results of LTT in vitro to PHA and $NiSO_4$*

Harvest of PHA took place on day 4 (96 hours) and harvest of $NiSO_4$ on day 5 (120 hours). The results of the LTT are given in counts per minute

Case	Date of patch test	Positive patch test to	Clinical manifestation at time of LTT	Date of LTT	PHA	LTT: $NiSO_4$-dilution factor 5 000	10000	20 000
(a) Patients with contact dermatitis and positive nickel patch test								
1	1974-05-?	Nickel, rubber	Subacute and chronic eczema of hands and feet	1976-11-22	44 442	4 328	4 279	3 609
2	1975-05-05	Nickel	Acute, dyshidrotic eczema of hands	1976-12-06	19 776	981	1 458	1 053
3	1976-12-01	Nickel	Subacute eczema of hands	1976-12-06	40 166	1 205	785	760
4	1976-12-07	Nickel	Acute, dyshidrotic eczema of hands	1976-12-13	49 372	3 332	4 075	3 366
5	1976-08-09	Nickel, Chromium, Cobalt, Paraphenylene-diamine	Acute, dyshidrotic eczema of hands	1977-01-10	32 601	2 755	2 428	1 376
6	1976-08-11	Nickel	Acute, dyshidrotic eczema of hands	1977-01-10	30 005	4 937	4 955	4 597
7	1976-11-01	Nickel	Acute and subacute eczema of back, arms and hands	1977-01-10	32 555	2 150	3 053	906
8	1977-01-06	Nickel	Acute, dyshidrotic eczema of feet	1977-01-10	23 491	2 894	3 481	2 975
(b) Patients with contact dermatitis and negative nickel patch test								
1	1976-11-17	–	Subacute and chronic eczema of hands and feet	1976-11-29	23 073	226	237	245
2	1976-10-29	Chromium, Cobalt		1976-11-29	34 259	374	552	378
3	1976-05-18	–	Subacute and chronic eczema of hands and feet	1976-11-29	47 223	346	326	360
4	1976-11-19	Balsam of Peru, tar, lanolin	Acute hypostatic eczema	1976-11-29	26 933	203	253	234
5	1976-12-01	Rubber, tar	Subacute eczema of hands and feet	1976-12-06	37 164	673	584	610
6	1976-10-19	Rubber, tar, Paraphenylene-diamine	Subacute eczema of hands and feet	1976-12-13	31 899	658	620	563
7	1976-12-06	–		1976-12-13	26 091	1 303	887	990
(c) Controls without contact dermatitis and with negative nickel patch test								
1	1976-12-07	–	None	1976-11-22	45 300	1 217	792	646
2	1976-12-03	–	Psoriasis	1976-11-29	52 528	952	559	401
3	1976-12-07	–	Psoriasis	1976-11-29	23 421	313	353	368
4	1976-12-07	–	Psoriasis	1976-12-06	29 445	1 149	618	978
5	1976-12-07	–	Leg ulcer	1976-12-06	31 482	871	1 184	685
6	1976-12-14	–	Tinea pedis	1976-12-13	52 077	1 560	1 707	1 227
7	1976-12-14	–	Psoriasis	1976-12-13	52 458	445	527	377
8	1977-01-11	–	None	1977-01-10	20 321	940	676	516
9	1977-01-11	–	Psoriasis	1977-01-10	27 030	968	1 241	544

METHODS

The LTT technique has been described elsewhere (9). Briefly, blood was drawn in equal volumes of heparinized RPMI-1640 (Gibco) with 25 mM Hepes buffer. Lymphocytes were isolated by gradient centrifugation using Lymphoprep (Nyco, Oslo), washed three times in RPMI-1640 with 5% pooled human serum. The cultures were set up in triplicate in tubes containing 10^5 cells in 0.5 ml RPMI-1640 with 25 mM Hepes, 15% pooled human serum, 15 IU heparin, 2400 IU penicillin, 500 μg

40 000	100 000	Control
2 558	1 226	374
1 612	1 161	540
761	510	260
1 557	2 598	1 270
1 108	1 399	244
4 211	3 413	589
1 290	1 269	252
1 942	1 631	364
222	198	226
347	602	422
293	430	283
250	215	212
838	329	307
722	867	533
1 331	1 149	1 611
657	380	368
557	1 307	486
385	234	292
993	897	468
649	733	436
1 437	875	1 104
337	382	272
627	307	440
586	386	343

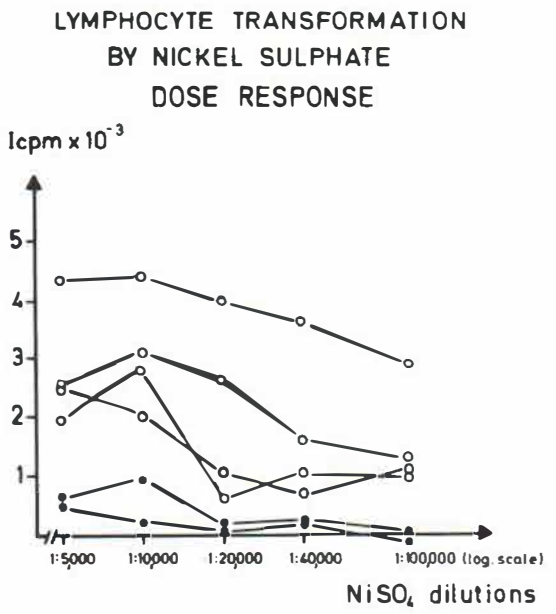

Fig. 1. Dose–response curves for 4 nickel-sensitive (○) and 2 nickel non-sensitive (●) persons. Icpm=increment counts per minute.

streptomycin base and 1.2 mmol glutamine. After addition of 50 μl mitogenic solution (either nickel sulphate, Phytohæmagglutinin (PHA), Concanavalin A (Con A), Pokeweed mitogen (PWM), Tuberculin (PPD) or *Candida albicans* extract (CA)), the cells were cultured in 5% CO_2 in humidified air at 37°C. Cultures with PHA, Con A or PWM were incubated for 96 hours, cultures with PPD or CA for 120 hours and nickel sulphate cultures for 120 hours. In the time course study, the nickel sulphate cultures were incubated for 96, 120, 144, 168, and 192 hours. To each tube 0.05 μCi of ^{14}C-thymidine (specific activity 0.25 mCi/mg; NEN Chemicals) was added 24 hours before harvesting on a Skatron semiautomatic harvester. The radioactivity was counted in a liquid scintillation counter. The stimulation was usually expressed as increment counts per minute (Icpm), i.e. counts per minute (cpm) in stimulated cultures minus cpm in unstimulated cultures. The medians of triplicates were used for calculation purposes. However, the figures in Table I are 'raw' cpm without subtraction of the controls.

Nickel sulphate. A stock solution of 1% nickel sulphate ($NiSO_4$, $7H_2O$; Merck No. 6725) in 0.9% saline was prepared. Additional dilutions were performed using RPMI-1640 as the diluent. The final concentration of nickel sulphate in the culture tubes ranged from 7.2×10^{-5} M to 3.6×10^{-6} (dilution 1 : 5 000 to 1 : 100 000).

The toxicity of the various concentrations of nickel sulphate was determined by incubating LTT-cultures from one patient and one control individual with both optimal concentration of PHA and nickel sulphate in the same culture tubes for 96 hours.

Investigation of the general responsiveness of patients and controls by the LTT was performed by stimulating cultures with PHA, Con A, PWM, PPD and CA.

The LTT was performed on five different occasions with the inclusion of 2 control individuals each time.

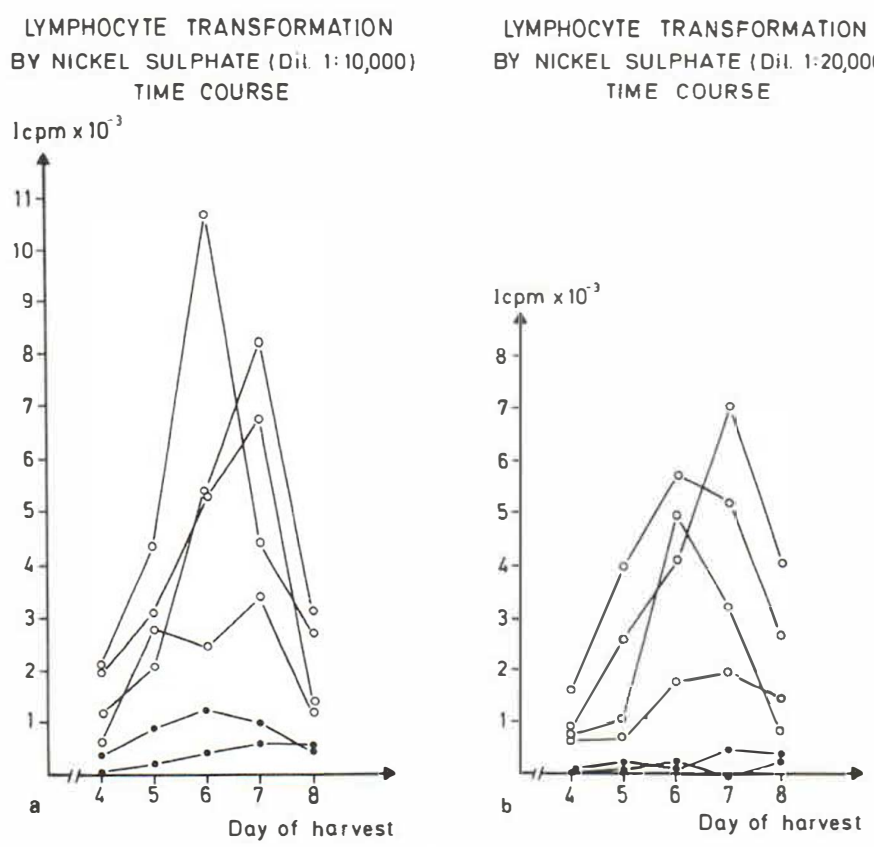

Fig. 2 a and b. Time course curves for 4 nickel-sensitive (○) and 2 nickel non-sensitive (●) persons. Mitogen: $NiSO_4$. Dilutions 1 : 10000 and 1 : 20000.

RESULTS

A. *Lymphocyte response to mitogens and antigens other than nickel*

It is evident from Table I that the three different groups of subjects responded equally well to PHA. This was true also of the responsiveness to PWM, Con A, and the antigens PPD and CA. When comparing the response of the 8 nickel-allergic patients with that of the 16 controls by means of the Mann-Whitney test, no significant deviations were found for any of these mitogens or antigens.

B. *Lymphocyte response to nickel sulphate*

Toxicity of nickel sulphate. When both PHA in optimal concentrations and nickel sulphate in concentrations of 3.6×10^{-3} M and 3.6×10^{-4} M were present in the cultures, no thymidine incorporation was found after 96 hours of incubation. However, normal responsiveness to PHA was found when nickel sulphate was present in concentrations between 3.6×10^{-5} M and 3.6×10^{-9} M. Both the patient and the control individual showed normal LTT responsiveness in vitro to PHA, Con A, PWM, PPD and CA.

Figs. 2*a* and 2*b* show a time-course study for 4 nickel allergic patients and 2 controls with lymphocytes harvested daily from day 4 (96 hours) to day 8 (192 hours). It appears the cells still show exponential growth on day five, while the maximum response was seen on day six or seven. Fig. 1 shows dose-response curves for 4 patients and 2 controls. Maximum responses occur with the dilutions 1 : 5000 and 1 : 10000. It appears from these figures and from Table I that the weakest responses were seen when the weakest nickel sulphate solutions were used.

It appears from Table I that 7 of the 8 patients with nickel contact dermatitis showed thymidine incorporation at one or more of the $NiSO_4$ concen-

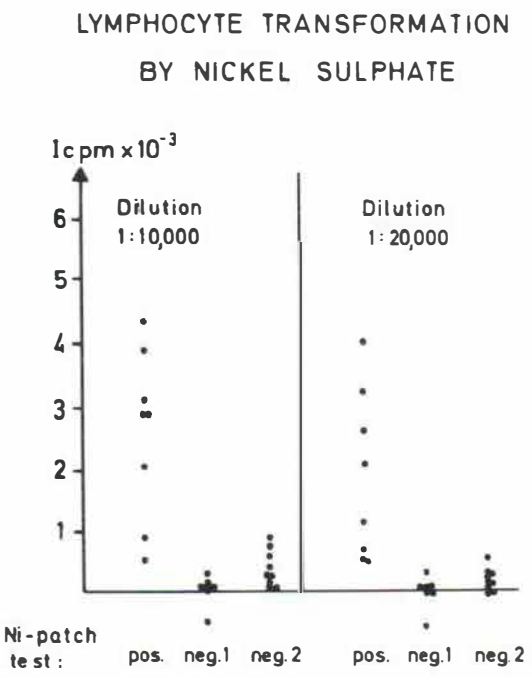

Fig. 3. Lymphocyte transformation in vitro to $NiSO_4$, dilution 1 : 10 000 and 1 : 20 000 in nickel-sensitive persons (pos.), nickel non-sensitive persons with active contact dermatitis (neg. 1) and nickel non-sensitive persons without contact dermatitis (neg. 2).

trations with cpm exceeding control cpm by more than 1 000. None of the subjects in the two control groups showed a similar response. In the group of nickel allergics, the dilutions 1 : 5 000 and 1 : 10 000 gave maximum stimulation except for patient no. 2. The dilutions 1 : 10 000 and 1 : 20 000 gave the clearest distinction between nickel-allergic patients and controls (Fig. 3). However, for all five dilutions of nickel sulphate used, there is a statistically significant ($p<0.001$, Mann-Whitney test) difference between the 8 patients with nickel hypersensitivity and the 16 controls.

Nevertheless, it is of interest to note that 3 of the controls showed significant responses (Icpm greater than 500) to nickel sulphate at the dilution 1 : 10 000. Moreover, these 3 individuals belonged to the group without history of contact dermatitis. They were all patch tested after LTT and all proved nickel patch test negative.

For those patients with nickel hypersensitivity there seems to be no correlation between the interval between patch testing and LTT and the degree of responsiveness to nickel sulphate.

DISCUSSION

The above results definitely confirm previous observations (3, 4, 5, 6, 7) that patients with a positive nickel patch test and a dermatitis compatible with nickel contact dermatitis respond more vigorously by LTT when their lymphocytes are challenged with nickel sulphate. Furthermore, none of our patients with contact dermatitis caused by other agents showed a significant response to nickel sulphate in the LTT. These observations provide sound evidence that the LTT with nickel sulphate may be used as an in vitro technique for the detection of nickel hypersensitivity. This may be valuable in cases of acute contact dermatitis in which patch testing may be impossible or undesirable. All the nickel-allergic patients included in this study had acute or subacute dermatitis. Studies designed to show how long the positive LTT persists after the skin lesions have subsided are in progress. The nickel-allergic patient who showed the weakest response in the LTT (no. 3, Table I), also had a peculiar reaction to the patch test which did not turn positive until 4 days after nickel was applied.

In some previous reports (1, 8) it has been claimed that nickel may cause non-specific lymphocyte transformation, and some of our results seem to support this notion. At least 3 patients without contact dermatitis (nos. 5, 6 and 9 in Table I) showed significant responses to nickel sulphate by the LTT. There is no explanation for this, but nickel sulphate could be a weak non-specific mitogen. Another possibility is that the LTT is a more sensitive test for nickel allergy than is patch testing and that nickel allergy may be a latent condition. However, it is worth noting that none of the patients with contact dermatitis due to other antigens showed more than borderline responses in the LTT. The difference between this control group and the other controls, however, is not statistically significant and may thus be due to chance.

The fact that all three groups responded equally well to PHA, Con A, and antigens other than nickel renders it unlikely that the high responsiveness of the nickel allergics to nickel seen in the LTT is due to a universal hyperresponsiveness in this category of patients.

In itself, it is a peculiar observation that the mere addition of a substance as simple as nickel sulphate can cause lymphocytes to transform. The nickel ion cannot be a complete antigen, but it might act as a

hapten. However, it is also possible that the nickel ion transforms other substances in the solution, e.g. serum proteins or cell surface components, into antigens. In any case, the results show that similar matter, whether a vehicle for nickel or capable of transformation by it, is present in the culture system. Unfortunately, the fact that it is so difficult to grow lymphocytes in serum-free media makes it hard to characterize the substances reacting with nickel.

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REFERENCES

1. Aspegren, N. & Rorsman, H.: Short term culture of leucocytes in nickel hypersensitivity. Acta Dermatovener (Stockholm) *42:* 412, 1962.
2. Fisher, A. A.: Contact Dermatitis. Lea & Febiger, Philadelphia, 1967.
3. Forman, L. & Alexander, S.: Nickel antibodies. Br J Dermatol *87:* 320, 1972.
4. Gimenez-Camarasa, J. M., Garcie-Calderson, P., Asensio, J. & De Moragas, J. M.: Lymphocyte transformation test in allergic contact nickel dermatitis. Br J Dermatol *92:* 9, 1975.
5. Hutchinson, F., Raffle, E. J. & MacLeod, T. M.: The specificity of lymphocyte transformation in vitro by nickel salts in nickel sensitive subjects. J Invest Dermatol *58:* 362, 1972.
6. MacLeod, T. M., Hutchinson, F. & Raffle, E. J.: The uptake of labelled thymidine by leucocytes of nickel sensitive patients, Br J Dermatol *82:* 487, 1970.
7. Millikan, L. E., Conway, B. A. & Foote, J. E.: In vitro studies of contact hypersensitivity: Lymphocyte transformation in nickel sensitivity. J Invest Dermatol *60:* 88, 1973.
8. Pappas, A., Orfanos, C. E. & Bertram, R.: Nonspecific lymphocyte transformation in vitro by nickel acetate. J Invest Dermatol *55:* 198, 1970.
9. Platz, P., Fog, T., Morling, N., Svejgaard, A., Sønderstrup, G., Ryder, L. P., Thomsen, M. & Jersild, C.: Immunological in vitro parameters in patients with multiple sclerosis and in normal individuals. Acta Pathol Microbiol Scand, sect. C, *84:* 501, 1976.

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