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LYMPHOCYTE TRANSFORMATION TEST IN PATIENTS WITH NICKEL SENSITIVITY: AN AID TO DIAGNOSIS

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Abstract. Peripheral blood lymphocytes from 16 patients with a positive patch test to 2.5% nickel sulphate ($NiSO_4$) and 18 healthy controls were tested by the lymphocyte transformation technique where $NiSO_4$ in six different concentrations was used and tested after various days of culture. Lymphocytes from all except one of the patients showed a significantly greater response than that of the controls. Lymphocyte transformation as measured by increased DNA synthesis seems to be a valuable tool for investigating the problems arising from false-positive or false-negative patch tests. With these data we have defined certain criteria for in vitro reactivity that should be fulfilled. Lymphocytes from controls responded non-specifically to high concentrations of $NiSO_4$. Cord blood lymphocytes from 4 newborn infants could also be activated by $NiSO_4$, thus confirming the assumption that $NiSO_4$ is a weak mitogen.

Key words: Nickel sensitivity; Lymphocyte stimulation; *In vitro* criteria

Nickel sensitivity is the most common cause of allergic metal dermatitis, particularly in women (3, 12). The diagnosis of nickel allergy is usually based on case history, clinical findings and results of patch testing. However, patch testing can give false-positive or false-negative results (4). Thus reliable in vitro methods are desirable to complement the in vivo tests. When using a lymphocyte stimulation test with nickel salts, both specific and non-specific stimulation have been reported (1, 6, 7, 8, 9, 10, 11, 14, 15). In this paper we present in vitro experiments with nickel sulphate, designed primarily to show whether the DNA synthesis test could be used to diagnose nickel allergy in vitro.

MATERIAL AND METHODS

Patients. Sixteen patients with a positive patch test to $NiSO_4$ were included in the study. They ranged in age from 25 to 63 years (mean 39.8 years). There were 15 women and one man. Five patients with positive patch tests to potassium dichromate ($K_2Cr_2O_7$) and/or cobalt chloride ($CoCl_2$) were also included. Their ages ranged from 48–77 years (mean 63.2 years). They were 3 women and 2 men.

Controls. Lymphocytes from altogether 18 age- and sex-matched healthy individuals without eczema or a case history of metal contact allergy were included in the experiments. Cord blood lymphocytes from 4 newborn infants were also tested.

Patch testing. The patch test was performed according to Pirilä (13). The test concentration for $NiSO_4$ was 2.5%, for $K_2Cr_2O_7$ 0.5% and for $CoCl_2$ 1%, all compounds in petrolatum. The test chamber was removed after 48 hours, 24 hours prior to reading. The test reactions were graded as follows:

\+ redness, palpable oedema
++ redness, oedema, papules
+++ redness, oedema, papules, vesicles

In vivo serial dilution test. This was performed using the following concentrations of $NiSO_4$ in distilled water: 1.25, 0.625, 0.312, 0.156, 0.078, 0.039, 0.019 and 0.009. The conditions and time of exposure and reading of the test were as mentioned above.

Preparation of $NiSO_4$ and PHA. A stock solution of 1% $NiSO_4 \cdot 6H_2O$ (Merk) was prepared in 0.9% sodium chloride solution. Six different concentrations of this antigen were prepared in modified Eagle's minimum essential medium (MEM) (Flow Laboratories, Irvine, Scotland) without serum, giving final concentrations in culture of 6.25, 12.5, 25, 50, 100 and 200 μg/ml. Phytohaemagglutinin (PHA) (Wellcome) was added to a final concentration of 50 μg/ml.

Assay of DNA synthesis. Lymphocytes were separated from heparinized blood by the rapid one-step Isopaque/Ficoll (Nyegaard AS, Oslo, Norway) procedure (2), washed three times in buffered saline and resuspended in MEM to which 1% HEPES buffer, 1% glutamine and 20% pooled human AB serum were added. Cultures were set up in triplicate in round-bottom microtitre plates (Nunc, Denmark), each well containing 1×10^5 cells in 0.1 ml medium. 0.1 ml of MEM (unstimulated culture), $NiSO_4$, or PHA in MEM without serum was added. The plates were then incubated at 37°C in humidified air containing 5% CO_2. To each well, 1 μCi of methyl [^{3}H]thymidine (Radiochemical Centre, Amersham, England, spec. act. 5 Ci/mmol) contained in 0.05 ml was added 24 hours before harvesting on fibreglass filters. Harvesting was done on days 1–10 in the case of cells from the patients, on days 1–8 for controls and on days 4–8 for the newborn infants.

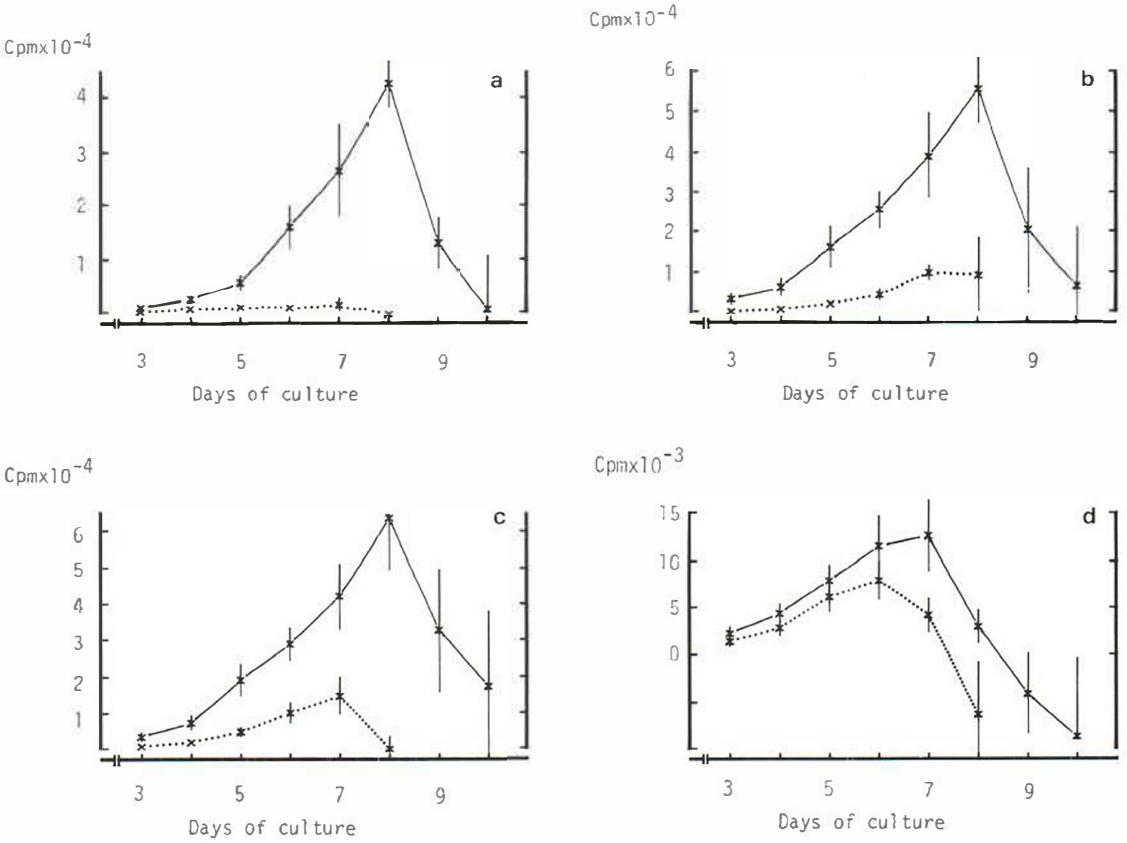

Fig. 1. (a–d) Time–response curves for peripheral blood lymphocytes from 15 patients with positive patch tests to $NiSO_4$ (×—×) and 15 controls (× . . . ×) using 6.25, 12.5, 25 and 50 μg $NiSO_4$/ml culture. The mean increment counts per minute and standard error values are plotted. Lymphocyte transformation by (*a*) 6.25 μg $NiSO_4$/ml, (*b*) 12.5 μg $NiSO_4$/ml, (*c*) 25 μg $NiSO_4$/ml, (*d*) 50 μg $NiSO_4$/ml.

using a Titertek cell harvester (Flow Laboratories, Irvine, Scotland). Filters were dried and then assayed in a liquid scintillation counter (Intertechnique, France). Results were expressed as increment counts per minute (Icpm) where the mean cpm of triplicates of unstimulated cultures were subtracted from the mean cpm of the cultures stimulated with $NiSO_4$ or PHA, and as stimulation indices (SI) where the mean cpm of stimulated cultures were divided by the mean cpm of unstimulated cultures.

Statistical method

The Student's *t*-test was used. *P* values of <0.05 were considered significant.

RESULTS

All the 16 patients showed a two plus reaction or more when patch tested with 2.5% $NiSO_4$. Nine of them participated in the in vivo serial dilution test. A test reaction of the skin could be read in 6 patients in 4–6 dilution steps but in 3 patients the serial dilution test was negative.

Lymphocytes of 15 patients (excluding No. 17, see below) with positive patch tests to $NiSO_4$ gave their highest response on day 8 of culture when stimulated with 6.25, 12.5 and 25 μg $NiSO_4$ (Fig. 1*a*, *b*, *c*) and on day 7 when stimulated with 50 μg/ml (Fig. 1*d*). Lymphocytes of controls showed a weak response to 6.25, 12.5 and 25 μg $NiSO_4$/ml, which was maximal on day 7 (Fig. 1*a*, *b*, *c*) while the peak response to 50 μg/ml was on day 6 (Fig. 1*d*). Responses of the patients' lymphocytes were significantly higher than those of the controls ($P<0.05$) when stimulated with 6.25, 12.5 and 25 μg but not with 50 μg. Neither patient nor control lymphocytes showed any response when stimulated with 100 μg and 200 μg $NiSO_4$/ml of culture. Responses of lymphocytes from individual patients

Table I. *Response (SI) of lymphocytes from 11 patients with positive patch tests to $NiSO_4$, $K_2Cr_2O_7$ and/or $CoCl_2$ and 4 control subjects, on day 6 of culture*

Expt. no.	Pat. no.	Background (cpm)	Concentration of $NiSO_4$ (μg/ml)				Clinical metal allergy
			6.25	12.5	25	50	
I	10	597	10.9	23.4	30.8	15.4	Ni
	11	1 838	17.5	20.3	23.2	12.6	Ni
	12	3 057	3.2	3.4	5.3	8.1	Cr & Co
	C[a]	1 707	2.5	1.9	8.2	7.4	–
II	13	1 547	7.5	10.5	13.2	8.4	Ni
	14	1 367	1.5	2.0	5.0	3.7	Cr
	15	960	1.3	1.9	4.3	5.5	Cr & Co
	16	4 222	9.1	10.6	13.9	10.8	Ni
	C	835	1.1	1.8	5.6	10.4	–
	C	2 213	1.2	1.2	3.7	5.8	–
III	18	2 820	8.1	9.2	11.8	3.0	Ni
	19	1 404	4.3	5.4	19.6	6.9	Cr
	20	319	3.5	2.9	9.3	17.4	Co
	21	1 547	26.8	28.0	33.9	10.7	Ni
	C	386	1.5	2.1	6.1	10.2	–

[a] C = control.

were almost always higher than control lymphocyte responses when tested simultaneously using 6.25, 12.5 and 25 μg $NiSO_4$/ml on various days of culture. From the above data, the following criteria were suggested and should all be fulfilled in order to define nickel allergy in vitro.

On day 5 of culture; the SI of the patient cells should be twice the SI (or more) of the healthy control cells simultaneously tested using 12.5 and 25 μg $NiSO_4$/ml. In addition, on day 6 of culture; the experimental SI should be at least twice the SI of the controls simultaneously tested using 6.25 μg $NiSO_4$/ml, and the SI of the patients should be more than 3 using 6.25 μg and more than 6 using 12.5 μg/ml.

To test the specificity of $NiSO_4$-induced activation, we stimulated lymphocytes from 5 patients with positive patch tests to $K_2Cr_2O_7$ and/or $CoCl_2$ with different concentrations of $NiSO_4$ for various days in culture. In these experiments we also included cells from 6 of the patients who were patch test positive to $NiSO_4$ as well as from healthy controls. The tests were performed on 3 different occasions (Table I). The response of lymphocytes from all the patients with positive patch tests to $K_2Cr_2O_7$ and/or $CoCl_2$ did not fulfil our criteria of in vitro nickel reactivity, thus confirming the specificity of the test.

Three of the patients who were patch test positive to 2.5% $NiSO_4$ were found to have a negative response to serial dilutions of the compound when applied in vivo. Case history and follow-up of these patients revealed that one of them (No. 17) was not nickel allergic, while the other 2 (Nos. 7 and 9) were. Their stimulation indices fit and confirm such a diagnosis (Table II).

Another important finding which demanded evaluation was that lymphocytes from our healthy controls also showed a weak response to stimulation with $NiSO_4$. In order to ascertain whether these findings reflect a low degree sensitization or are due to a non-specific mitogenic effect of $NiSO_4$, cord blood lymphocytes were stimulated with various concentrations of $NiSO_4$ on days 4–8 of culture. Cord blood lymphocytes could indeed be activated by $NiSO_4$ and the stimulation was more pronounced at higher concentrations (Table III). This demonstrates that $NiSO_4$ is a weak mitogen, which might also explain the low degree stimulation observed with cells from healthy controls.

Lymphocytes from all patients and controls responded to PHA. On day 4 of culture, patient and control lymphocyte responses were within the range of 54–223 and 58–204 cpm $\times 10^{-3}$ respectively.

DISCUSSION

Sensitivity against nickel is a commonly occurring contact allergy affecting mostly women. In a recent-

Table II. *Response (SI) of lymphocytes from 3 patients with a positive patch test to 2.5% $NiSO_4$ (in vivo dilution test negative) and 3 controls on day 6 of culture*

Pat. no.	Background (cpm)	Concentration of $NiSO_4$ (μg/ml)				Clinical metal allergy
		6.25	12.5	25	50	
7	845	9.5	17.9	23.1	6.0	Ni
C[a]	597	nd[b]	3.7	6.6	13.8	–
9	2 568	6.0	10.5	13.1	8.6	Ni
C	1 695	0.7	1.7	5.0	4.9	–
17	1 600	1.5	1.9	2.1	5.6	–
C	386	1.5	2.1	6.1	10.2	–

[a] C = control.
[b] nd = not done.

ly performed investigation of a Finnish population it was observed that as many as 8% of the females were patch test positive to nickel and that only one-third of them had active eczema (12). Some investigators have tried to use the lymphocyte transformation test to aid in the diagnosis of nickel allergy (10, 14, 15) but none gave criteria that define nickel sensitivity in vitro. On the basis of our study we are able to provide such criteria.

One patient (No. 17) had a positive patch test to 2.5% $NiSO_4$ but his lymphocyte response did not fulfil our criteria. Case history and follow-up of this patient showed that the in vivo reaction was a false-positive. Two other patients (Nos. 7 and 9) also had positive patch tests to 2.5% $NiSO_4$ but the diagnosis was kept provisional, since the in vivo serial dilution tests were negative. By following up these 2 patients the diagnosis of nickel sensitivity was established, which was also independently reached by using our in vitro criteria. In all such

Table III. *^{3}H-thymidine uptake (cpm×10^{-3}) by cord blood lymphocytes of 4 newborn infants and peripheral blood lymphocytes of 3 healthy controls and one nickel-allergic subject, following stimulation by $NiSO_4$, on days 5 and 6 of culture*

Stimulation indices are shown in parentheses

Subjects[a]	Days	Background	Concentration of $NiSO_4$ (μg/ml)				PHA (μg/ml)
			6.25	12.5	25	50	50
1	5	1.9	2.1 (1.1)	2.5 (1.4)	6.6 (3.5)	8.7 (4.7)	28.9 (15.6)
	6	2.4	3.5 (1.5)	3.7 (1.6)	3.1 (1.3)	12.6 (5.3)	27.2 (11.5)
2	5	15.1	16.8 (1.1)	19.5 (1.3)	20.7 (1.4)	27.4 (1.8)	20.0 (1.3)
	6	29.7	32.3 (1.1)	35.8 (1.2)	43.6 (1.5)	35.7 (1.2)	13.8 (0.5)
3	5	13.3	12.7 (1.0)	15.3 (1.1)	19.0 (1.4)	27.5 (2.1)	45.8 (3.4)
	6	10.1	12.2 (1.2)	17.3 (1.7)	7.3 (0.7)	13.2 (1.3)	55.7 (5.5)
4	5	9.1	11.9 (1.3)	14.4 (1.6)	20.4 (2.2)	29.4 (3.2)	31.4 (3.5)
	6	17.0	23.3 (1.4)	27.9 (1.6)	20.2 (1.2)	20.0 (1.2)	24.0 (1.4)
C1	5	2.0	2.2 (1.1)	2.3 (1.2)	5.6 (2.8)	11.0 (5.5)	87.8 (44.1)
	6	3.6	6.1 (1.7)	9.5 (2.7)	19.8 (5.5)	24.3 (6.8)	18.2 (5.1)
C2	5	0.9	1.6 (1.8)	2.0 (2.2)	3.9 (4.2)	14.1 (15.3)	40.8 (44.5)
	6	1.0	3.7 (3.7)	5.5 (5.5)	10.6 (10.6)	28.7 (28.7)	21.7 (21.7)
C3	5	0.8	0.8 (1.1)	0.4 (0.5)	0.3 (0.4)	2.2 (2.8)	75.7 (99.4)
	6	0.6	0.4 (0.8)	0.5 (1.0)	0.5 (0.8)	4.0 (7.3)	98.5 (179.4)
21	5	2.8	18.1 (6.6)	28.0 (10.1)	27.6 (10.0)	40.3 (14.5)	70.7 (25.5)
	6	3.5	48.4 (14.0)	54.7 (15.8)	62.9 (18.0)	61.6 (17.8)	29.4 (8.5)

[a] 1–4: newborn infants.
C1–C3: healthy adults.
21: nickel-allergic subject.

instances the in vitro DNA synthesis test would be of great help in confirming the diagnosis without exposing the patients to nickel salt.

In this study it was also shown that the response of the nickel patch test positive patient lymphocytes was specific, as was demonstrated by the weaker responses of lymphocytes of 5 patients with positive patch tests to $K_2Cr_2O_7$ and/or $CoCl_2$. Although none of the latter 5 patients could fulfil all the criteria, it was noted that one of them (No. 19) had reactions very close to those suggested. Case history revealed that this patient was regularly exposed to many metal salts including $NiSO_4$. This patient may be in a latent stage of nickel allergy and further follow-up would prove or disprove such an assumption.

Some authors (1, 11) have found that nickel may lead to non-specific transformation of lymphocytes and some have suggested the possibility that nickel could be a weak non-specific mitogen (15, 16) but they gave no evidence in support. In the present investigation, lymphocytes from healthy controls also showed a non-specific transformation induced by $NiSO_4$. For this reason we tested cord blood lymphocytes of 4 newborn infants for any mitogenic effect of $NiSO_4$, together with lymphocytes from 3 healthy adults and one nickel-sensitive patient (No. 21). Our results confirm the assumption that $NiSO_4$ is a weak mitogen.

Finally, it is quite clear that lymphocyte transformation measured by the DNA synthesis test is a reliable method to diagnose nickel allergy without exposing the patients to the hazards of patch testing. Work is in progress to find out whether this test could be applied to diagnose sensitivity to other metals such as cobalt and chromium. We have also started investigating the role of cytotoxic T-lymphocytes in the pathogenesis of nickel allergy and it seems at present that these lymphocytes show some cytotoxicity following primary cultures in vitro. Future studies will also try to elucidate whether nickel allergy is HLA-associated, whether T cell activation to nickel is HLA-restricted and furthermore we shall try to define the target cell for both specific and non-specific activation by nickel salts.

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