Metal Allergy in Cashiers

An In Vitro and In Vivo Study for the Presence of Metal Allergy

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Thirty healthy cashiers continuously exposed to nickel in coins were tested in vivo and in vitro for the presence of metal contact allergy. A traditional epicutaneous test and lymphocyte transformation test were used. We tested for nickel, cobalt and chromium sensitivity. Seven of the 30 cashiers were patch test positive and 3 were in vitro positive to nickel sulphate. Two were in vivo positive to cobalt and only one in vitro positive. None was chromium allergic. There was no correlation between the exposure time and the lymphocyte response towards nickel. The presence of pierced and non-pierced ear lobes was noted with and without eczema in conjunction with the wearing of ear-rings containing nickel. The lymphocyte reactivity showed no significant difference between these groups. Only 5 out of the 12 with ear lobe dermatitis were patch test positive towards nickel. The data suggest that nickel as test substance or released from nickel-containing jewellery can evoke a cutaneous response which is not always associated with allergy. *Key words: Nickel sensitivity; Patch testing.* (Received May 1, 1987.)

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In recent years we have endeavoured to establish optimal conditions for the in vitro stimulation of lymphocytes with metal salts from nickel, cobalt and chromium allergic individuals (1, 2, 3). We consider the in vitro activation of lymphocytes to be a reliable method. However, the correlation between in vivo and in vitro response has not yet been exhaustively investigated. Qualitatively, there is a fair degree of agreement when comparing the two ways of diagnosing metal allergic patients. In the case of nickel and cobalt, 78% of patients with a positive patch test, peripheral blood lymphocytes give positive responses in vitro. The figure for chromium is 65% (4).

On the other hand, no agreement was found in a quantitative comparison of the cutaneous and lymphocyte responses (5). There are several conceivable reasons for this. One could be the way in which subjects are selected. In earlier reports our material consisted of patients who had consulted a dermatologist because of eczema. In consequence, an increased unspecific reactivity of the whole skin might be anticipated, thus giving an increased number of false-positive reactions when patch tested (4, 5, 6). This could explain the disparity.

The purpose of the present investigation was threefold:

to apply the in vitro method to a group of individuals who are continuously exposed to nickel in coins through their occupation as cashiers;

to further evaluate the in vitro and in vivo response in subjects without eczema;

to assess the frequency of dermatitis of the pierced ear lobe in subjects wearing ear-rings containing nickel, vis-à-vis the results of testing in vivo and in vitro with nickel sulphate.

MATERIALS AND METHODS

Subjects

Cashiers: Thirty female cashiers (median age 37, mean 38, range 20–61 years) were tested for nickel, cobalt and chromium sensitivity in vivo and in vitro. They were employed in stores, banks and post offices, and were thus continuously exposed to nickel in coins during their 40-hour working week. Contact with the nickel was dry. 'Silver coins' in circulation in Sweden have a nickel content which varies between 25 and 35%. The subjects were recruited for the study when on duty and were not patients of any dermatologist.

The number of subjects with pierced ears was noted, both of those with and without dermatitis caused by wearing ear rings containing nickel. All were healthy. None had eczema of any kind at the time of investigation. Questions were asked regarding cutaneous symptoms following direct contact with jeans buttons, clasps, nickel-containing jewellery and suspenders. An affirmative answer will be referred to as positive history.

Controls: Mononuclear cells from 15 controls (13 females and 2 men) matched by age were used in the tissue culture experiments. None was suffering from any skin disease, nor had they any previous history of eczema or metal contact allergy. The control subjects were only tested in vitro.

In vivo testing

The patch test was done as before (7, 8). The cashiers were simultaneously tested with the following four substances in diminishing concentrations (vehicle = petrolatum) (Epicon Oy, Helsinki, Finland).

Nickel sulphate: 5%, 2.5%, 1.0%, 0.32%, 0.1%, 0.032%, 0.010%
Cobalt chloride: 3.2%, 1.0%, 0.32%, 0.0032%
Chromium chloride (trivalent): 5%, 2%, 1% and 0.5%
Dichromate (hexavalent): 1.0%, 0.5%, 0.32%, 0.10%, 0.032% and 0.010%.

The positive cutaneous reactions were graded as follows (7):

0	no reaction
+	redness, palpable edema
++	redness, edema, papules
+++	redness, edema, papules, vesicles

The highest concentration has been designated dilution step I, the next step II, and so on. All cutaneous reactions found will be presented.

In vitro testing

The in vitro experiments were executed as before and only a brief description will be given (1, 2, 3).

Three dilutions of the metal salts were used in culture:

$NiSO_4$ (Merck): 6.25, 12.5 and 25 μg/ml
$CrCl_3$. 6 H_2O (trivalent) (BDH Chemicals Ltd, Poole, England): 25, 50 and 100 μg/ml
$K_2Cr_2O_7$ (hexavalent) (Merck, Darmstadt, Germany) 0.1, 0.2 and 0.4 μg/ml
$CoCl_2$. 6 H_2O (Merck, Germany): 1.5, 3.125 and 6.25 μg/ml.

The mononuclear cells of the cashiers and controls were tested with PHA (phytohaemagglutinin) 50 μg/ml as positive control. Cell cultures with medium served as background control.

Assay of DNA synthesis

Mononuclear cells were separated (9) from heparinized peripheral blood, washed and resuspended in modified Eagle's minimum essential medium (MEM) (Flow Laboratories, Irvine, Scotland) 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. Solutions of the metal salts and PHA were added to final concentrations as stated.

To each well, 1 μCi of methyl [^{3}H]thymidine (Radiochemical Centre, Amersham, England; spec. act. 5 Ci/mmol) was added 24 h before harvesting on fibreglass filters. Nickel and cobalt containing cultures were harvested on days 5 and 6; chromium cultures on days 6, 7 and 8. Filters were dried and assayed in a liquid scintillation counter.

Results were expressed either 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 the

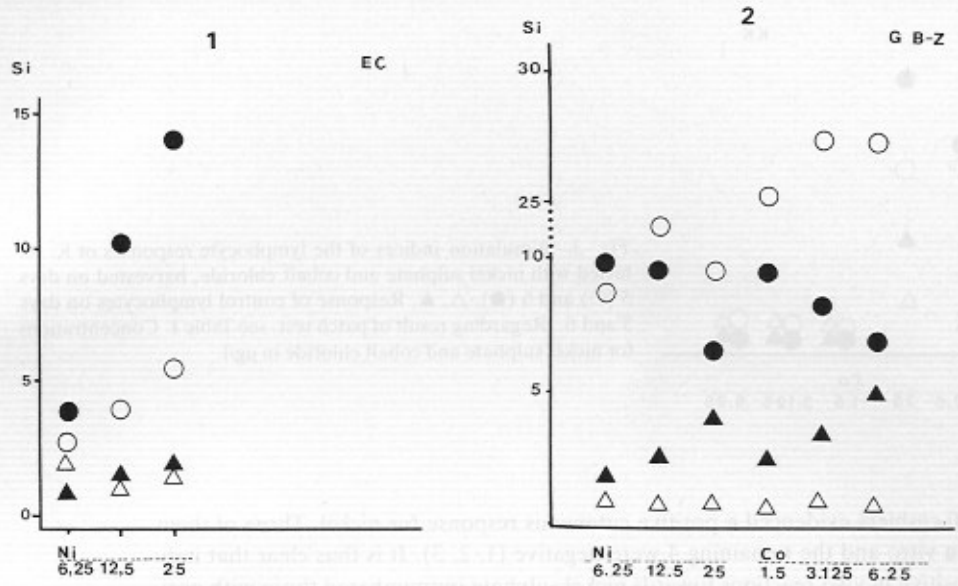

Fig. 1. Stimulation indices of the lymphocytes from individual EC tested against nickel sulphate in various concentrations harvested on day 5 (○) and 6 (●). The concentrations of the compound (in µg/l) are given. △, Response of control cells on day 5; ▲, responses on day 6. For patch test response and history, see Table I.

Fig. 2. Stimulation indices of the lymphocytes of G. B.-Z. tested against nickel sulphate and cobalt chloride in various concentrations harvested on day 5 (○) and 6 (●). Concentrations used are given in µg/ml. △, ▲, Responses of controls on days 5 and 6 respectively for each concentration. Regarding patch tests with nickel sulphate and cobalt chloride, see Table I.

metal compounds; or as stimulation indices (SI) where the mean cpm of the stimulated cultures were divided by the mean cpm of the background.

Previously defined criteria were applied when assessing the in vitro sensitivity of the subjects (1, 2, 3).

Statistical methods

Correlation analysis and Kruskal-Wallis test of differences.

Table I. *Results of the in vivo and in vitro tests*

The figures in the columns indicate a positive patch test reaction up to the given dilution step. Figures in parentheses indicate 1+ reactions at the given dilution step. E. C. showed only 1+ in dilution steps I and II. Regarding in vitro positive individuals and those with positive nickel history, see Materials and Methods

Subject	Exposure time	History of Ni-sensitivity	In vivo pos				In vitro pos			
			Ni	Co	Cr+6	Cr+3	Ni	Co	Cr+6	Cr+3
E. J.	2	+	1	–	–	–	–	–	–	–
E. C.	33	–	(2)	–	–	–	+	–	–	–
K. K.	4	+	6	3 (4)	–	–	+	–	–	–
M.-A. B.	8	–	1	–	–	–				
G. B.-Z.	3	+	6	6	–	–	+	+	–	–
S. B.	5	–	2	–	–	–	–	–	–	–
M. L.	4	–	1	–	–	–	–	–	–	–

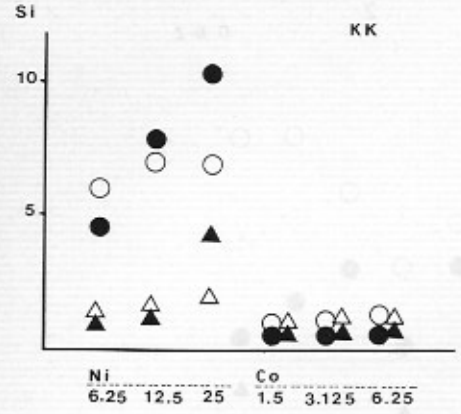

Fig. 3. Stimulation indices of the lymphocyte responses of K. K. tested with nickel sulphate and cobalt chloride, harvested on days 5 (○) and 6 (●). △, ▲, Response of control lymphocytes on days 5 and 6. Regarding result of patch test, see Table I. Concentrations for nickel sulphate and cobalt chloride in µg/l.

RESULTS

Seven of the 30 cashiers evidenced a positive cutaneous response for nickel. Three of them were positive in vitro and the remaining 4 were negative (1, 2, 3). It is thus clear that individuals with positive in vivo reactions towards nickel sulphate outnumbered those with positive lymphocyte responses. These 3 subjects deserve further attention.

E. C. had been working for 33 years with no anamnesis of nickel sensitivity. She had a weak positive (+1) cutaneous reaction for 5% and 2.5% $NiSO_4$ but was strongly positive in vitro (Table I, Figs. 1, 4). She was negative for cobalt in both test systems. Two of the others (G. B.-Z. and K. K.) had a positive patch test for nickel and cobalt (Table I, Figs. 2, 3). Both of them reported a positive nickel history. Their in vitro reaction for nickel was positive. However, K. K. was negative in vitro for cobalt, whereas G. B.-Z. was positive. These results were confirmed in two separate experiments.

In the chromium test, all the cashiers were negative in vivo as well as in vitro.

Fig. 4 shows the correlation between the exposure time (number of years employed as cashier) for nickel in coins and the peak of the in vitro responses. No correlation could be

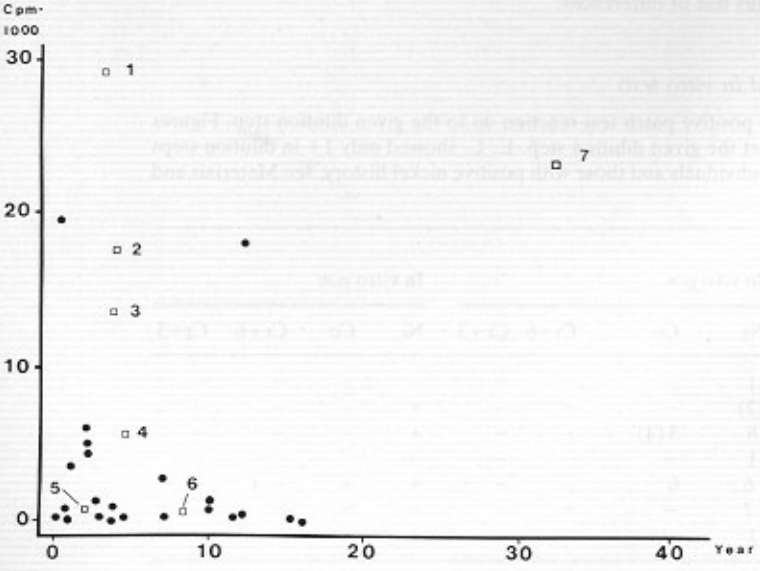

Fig. 4. Correlation between lymphocyte response to 12.5 µg nickel sulphate and exposure time (in years) on day 6. No correlation is found for any concentration of $NiSO_4$ used when harvested on days 5 and 6 (r ranging from 0.0–0.3). Values on X-axis represent years and on Y-axis, increments in cpm. □, Patch test positive individuals (see Table I). 1=G. B.-Z.; 2=M. L.; 4=S. B.; 5=E. J.; 6=A.-M. B.; 7=E. C.

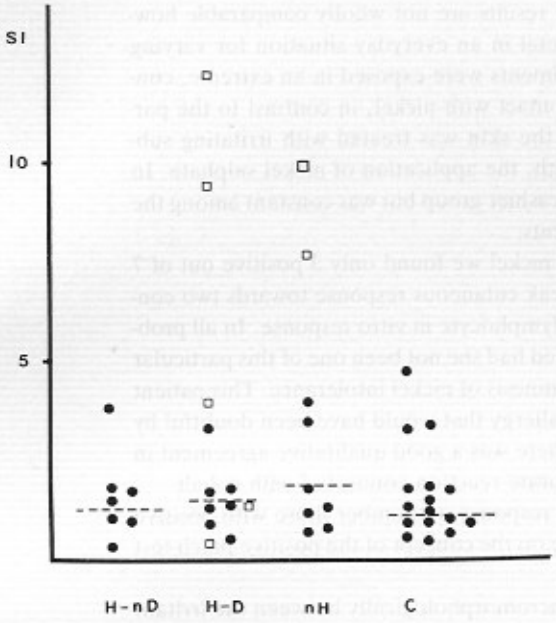

Fig. 5. The lymphocyte response to 12.5 µg/l $NiSO_4$ on day 6. The cashiers are divided into three groups according to the presence or absence of dermatitis in the vicinity of the pierced hole of the ear lobe and wearing ear-rings containing nickel. *H–nD*, Hole–no Dermatitis; *H–D*, Hole–Dermatitis; *O–H*, no Hole; *C*, control subjects. ---, Mean values of each group; □, patch test positive subjects (see Table I).

seen for any concentration of nickel sulphate. Six out of 7 in vivo positive subjects had been employed for less than 10 years. The individual (E. C.) with the longest period of employment showed high (but not the highest) lymphocyte responses.

Fig. 5 shows the lymphocyte response towards 12.5 µg of nickel sulphate on day 6 plotted in three columns according to the presence or absence of dermatitis around the hole of the pierced ear lobe (evaluated by history) when exposed to ear-rings containing nickel, and those with non-pierced ears. No statistically significant differences were found in the lymphocyte reactivity between the groups. This applies to all concentrations of $NiSO_4$ used. Only 5 out of the 12 with dermatitis had a positive patch test. Three had SIs of less than five for the data shown and were definitely not in vitro positive. Thus, with nickel sulphate, more than half of those having dermatitis were patch test negative and their in vitro responses were also low.

Most of the lymphocytes of the healthy control subjects responded in all experiments with SIs lower than five and none fulfilled the criteria for positive in vitro response.

DISCUSSION

In the present paper we have shown that about 20% (7 out of 30) of the cashiers were sensitized to nickel sulphate according to the patch test technique. As they are continuously exposed to nickel in coins during their working hours, a greater proportion could have been expected. The sensitizing capacity of nickel has earlier been investigated by Vanderberg & Epstein in 1963 (10). Allergy was induced in male volunteers by the application of 25% nickel sulphate solution together with 0.1% sodiumlauryl sulphate, after pretreatment of the skin by freezing. This procedure was repeated on three occasions and 9% (16 out of 172) became allergic (10). In 1966 Kligman (11) succeeded in producing allergy in half (12 out of 25) the subjects by using the maximization technique employing 10% nickel sulphate for induction. Similar results were obtained in guinea pig maximization tests (12).

The frequency of nickel allergy among cashiers found in our study thus falls in between the

data obtained by controlled experimentation. The results are not wholly comparable however, since the cashiers were exposed to nickel metal in an everyday situation for varying periods of time, while those included in the experiments were exposed in an extreme, controlled way. The cashiers were submitted to dry contact with nickel, in contrast to the participants in the experimental series. In the latter the skin was treated with irritating substances or procedures before, or in conjunction with, the application of nickel sulphate. In addition, the exposure time for nickel varied in the cashier group but was constant among the subjects participating in the sensitization experiments.

However, when evaluating the in vitro test with nickel we found only 3 positive out of 7 in vivo positive subjects. One had an extremely weak cutaneous response towards two concentrations of nickel sulphate but showed a strong lymphocyte in vitro response. In all probability her weak reaction would have been overlooked had she not been one of this particular group of test subjects. Furthermore she had no anamnesis of nickel intolerance. This patient exemplifies that in vitro testing can confirm metal allergy that would have been doubtful by routine patch testing. With regard to the other 2, there was a good qualitative agreement in vivo and in vitro linked to nickel, but quite a disparate reaction connected with cobalt.

Taken together, subjects with positive cutaneous response outnumber those with positive in vitro responses. What implication might this have on the concept of the positive patch test response as a representative of contact allergy?

Criteria have been formulated to differentiate macromorphologically between the irritant and allergic type of reaction (4, 6). These responses seem to be well defined but a strict subdivision seems to be deceptive.

In order to refine the patch test technique and devise a means to differentiate between the two cutaneous response patterns the test substances are used in diminishing concentrations (5, 13, 14). An allergic response is said to taper off along with the decreasing concentrations and an irritant reaction will disappear abruptly after one or more dilution steps.

Light microscopy investigation of skin biopsies, from sites where allergic and irritative responses have been provoked, have not allowed of any differentiation (15, 16). Nor have immunohistochemical analyses of the cellular infiltrates of a test area helped clarify the question (17, 18).

Moreover, recent data (19, 20) have shown that the majority of biopsies from 'allergic' reactions contain keratinocytes expressing HLA-DR antigens on their surface, which is not the case for the 'irritant' type of reaction. This may mean that the lymphocytes of the infiltrate in the two situations are differently activated, which could be an aid in differentiating between the two in vivo reaction patterns of the skin. Support for this idea has recently been published (21).

The disagreement between the two test systems is further emphasized by our results on skin reaction to direct contact with metal containing nickel, as assessed by careful anamnesis. The distribution of the lymphocyte responses of subjects with dermatitis around the hole in the ear lobe arising from wearing ear-rings containing nickel is shown in Fig. 5. The patch test was positive for nickel sulphate for fewer than the 50%. The lymphocyte response towards nickel was negative in most of the subjects. The findings agree with those of Widström et al. (22).

This implies that nickel ions as a test substance and nickel as a metal on the skin can provoke an inflammatory reaction which is not accompanied by sensitization, as measured in our proliferative in vitro test system. The inflammatory response can thus be subdivided into two patterns: one demonstrating the presence of sensitized lymphocytes in the peripheral blood, while the other does not. It is therefore tempting to extrapolate that the former type of cutaneous response provoked by nickel is an allergic, and the latter an irritant, type of reaction.

This leads us to discuss the recruitment of cells to the test site in the cases of irritatant or allergic reactions. We believe that nickel initially produces an irritant cutaneous reaction by direct effect on epidermal cell components, resulting in the release of chemoattractants causing accumulation of lymphocytes. If sensitized lymphocytes are present in the peripheral blood, the cell infiltrate will contain specific memory cells which are further activated by nickel sulphate which causes production and release of γ-interferon inducing expression of HLA-DR antigens on keratinocytes (19, 20, 24). In the other situation the lymphocytes would be attracted without accompanying specific lymphocytes, resulting in no augmentation of the inflammatory response and no subsequent HLA-DR expression. Since mediators have been identified as being released from the epidermal cells, a reciprocal interaction between the involved cell types is likely (23, 24).

An interesting finding is the lack of correlation between the exposure time to nickel in coins and the lymphocyte proliferative response. We found in vitro high responders with only a few years of professional contact with the metal as well as among those with many years' contact. It is therefore impossible to conclude that continuous contact with nickel in coins is the only factor that causes nickel allergy. Parameters other than those related associated with the cashier's occupation must therefore be involved in the sensitization process.

Finally, we conclude that the combination of the cutaneous response with the in vitro activation of lymphocytes will increase our knowledge and help us to elucidate the mechanism behind the cutaneous reaction elicited by either an allergen or irritant.

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