

THE LEUKOCYTE MIGRATION TEST IN NICKEL CONTACT DERMATITIS

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Abstract. Ten patients with contact dermatitis to nickel, confirmed by patch testing, were investigated by the leukocyte migration test. Specific inhibition of leukocyte migration was obtained with nickel protein complexes. Bovine albumin, human albumin and human epidermis protein were used as carrier proteins in the complexes. Specific inhibition of leukocytes from patients with positive patch test to nickel could not be demonstrated with the hapten alone.

Key words: Leukocyte migration test; Contact dermatitis; Nickel: Nickel allergens

The diagnosis of allergic contact dermatitis in patients with eczema is usually established by a positive patch test to the suspected antigens. A risk of sensitization due to the procedure does exist, however, and an exacerbation of the dermatitis can be seen. The advantages of *in vitro* testing are obvious. Since the primary reactive cell in contact hypersensitivity is the lymphocyte, testing for contact hypersensitivity may be done by using lymphocytes in the test system. Several workers have used the lymphocyte transformation test for detecting contact hypersensitivity to nickel (7, 8, 10, 11, 12). Another test, the leukocyte migration test (LMT), has been proposed as an *in vitro* parameter of delayed hypersensitivity (3). This test has been used successfully to detect contact hypersensitivity to chromium (15).

In the present study the LMT in the modification of Soeborg & Bendixen (3) was used in an attempt to demonstrate specific inhibition of leukocyte migration from patients with contact allergy to nickel. The antigens consisted of nickel protein complexes. As carrier proteins, bovine albumin, human albumin and human epidermis protein were used. Also, NiCl_2 was used alone without proteins in the test.

MATERIAL AND METHODS

The leukocyte migration test (LMT) was carried out in 10 patients with positive patch test to nickel (nickel sulphate 2% in petrolatum) and in 10 control patients without any history of eczema who were attending our clinic because of venereal disease. None of the patients had shown positive patch test reactions to other compounds. All had been tested with standard test series of the International Contact Dermatitis Group (5). They all had typical nickel contact dermatitis, none of them showed pompholyx-like eruptions, there was no suspicion of other allergic conditions, none of the patients were atopic and none of them received drugs systemically.

The LMT was carried out as previously described (15). Heparinized blood was obtained from patients as well as from controls and allowed to sediment spontaneously for 1 hour at 37°C. The white blood cells were removed and washed 3 times in Hank's balanced salt solution. The cell suspensions were then transferred to capillary tubes and the leukocytes allowed to migrate for 24 hours in the culture chambers. Identical sets of culture chambers were set up for controls and patients. 3 control cultures

Table 1. *Leukocyte migration indices in patients with contact allergy to nickel and in normal human subjects.*

Bovine albumin was used as antigen in a concentration of 15 mg/ml

Patients with positive patch test to nickel (migration index)	Controls (migration index)
1.46	1.54
1.62	1.54
1.25	0.99
1.53	1.63
2.42	1.22
1.07	2.20
1.41	1.65
1.19	1.13
	1.49
Mean	1.49
S.E.M. \pm 0.15	0.12

Table II. *Leukocyte migration indices in patients with contact allergy to nickel and in normal human subjects*

NiCl₂ was used as antigen in a concentration of 200 µg/ml

Patients with positive patch test to nickel (migration index)	Controls (migration index)
0.48	0.57
0.57	0.57
0.67	0.58
0.70	0.66
0.74	0.67
0.84	0.67
0.84	0.86
0.87	0.99
0.91	1.01
0.95	1.11
1.00	1.50
1.25	
Mean 0.84	0.84
S.E.M. ± 0.06	0.09

within each set without antigen, filled with TC medium 199 (Difco), and triplicate cultures for each antigen. After 20 hours the migration areas of the leukocytes around the openings of the capillary tubes were measured in a projection microscope. The average migration areas for three identical cultures containing antigen, Mx, and TC medium alone, Mo, respectively determined the migration index, Mi: $Mi = Mx/Mo$. A value of less than 1.0 indicates inhibition of migration due to the added antigen.

Several antigens were used in the test. NiCl₂ was used in a concentration of 200 µg/ml and bovine albumin (Sigma) in a concentration of 15 mg/ml in the culture chambers. In a preliminary investigation the above-mentioned concentrations had shown no or only slight inhibition of the outgrowth of leukocytes when compared with control cultures in control persons (15). Bovine albumin has the ability to bind NiCl₂ in a reversible manner, the concentration of bound NiCl₂ depending on the concentration of free nickel in the solution (4). Accordingly, a complex of bovine albumin, 15 mg/ml, and NiCl₂ 1:50 was made, the estimated concentration of free nickel in the solution being approximately 200 µg/ml of NiCl₂. No similar data concerning the binding capacity of human albumin and human epidermis protein could be obtained. In the above-mentioned preliminary investigation (15) the non-inhibitory concentrations of human albumin and human epidermis protein to normal leukocytes were estimated and found to be 3.5 mg/ml of human albumin and 0.05 mg protein/ml of epidermis protein. Complexes of NiCl₂ and human albumin 1:125, and of NiCl₂ and human epidermis protein 1:2 induced only slight inhibition of normal leukocytes and were chosen as antigens in LMT (15). All nickel protein complexes were stored at 5°C for a minimum of 3 days before use. pH was adjusted to 7.3. Epidermis was separated from dermis by heating 10 minutes at 60°C (14) and thereafter homogenized in leukocyte culture medium (Difco 199 TC medium) using a motor-driven glass homogenizer. The homogenate was

stored at 5°C overnight and then centrifuged at 1000 g for 30 minutes. The protein concentration of the supernatant was estimated by the method of Lowry (9).

RESULTS

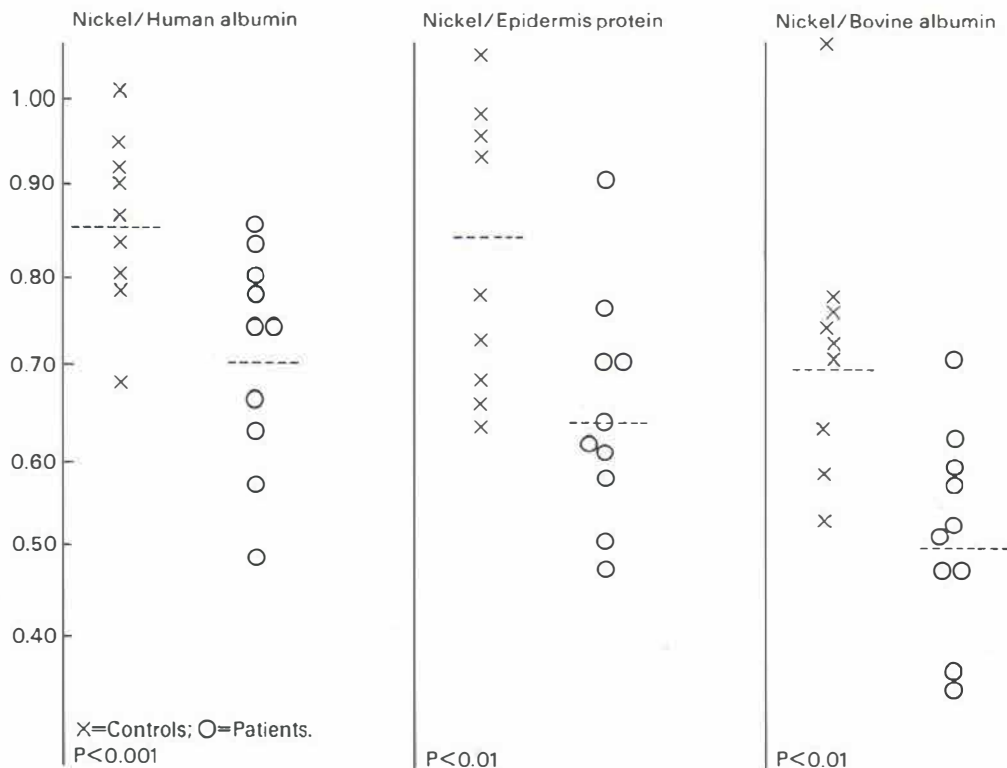
Table I shows migration indices from 8 patients with contact allergy to nickel and 9 controls where bovine albumin without addition of nickel was used as antigen. No difference could be demonstrated between results from patients and controls. Bovine albumin had a stimulatory effect on the migration of leukocytes in patients as well as controls. NiCl₂ alone was also used as antigen in the test and the results appear in Table II. No difference was found between mean values in patients and controls. In Table III, migration indices are shown using a complex of NiCl₂ and bovine albumin as antigen. The difference between the mean values is significant ($p < 0.001$). The mean value for patients was 0.49 and for controls, 0.70. Fig. 1 further illustrates these results together with the results when using complexes of NiCl₂ and human albumin and human epidermis protein. All antigens induced specific inhibition of migration of leukocytes from patients with contact allergy to nickel. When using NiCl₂ and human albumin the mean value for patients was 0.71 and 0.85 for controls. With nickel/epidermis protein as antigen the mean value for the pa-

Table III. *Leukocyte migration indices in patients with contact allergy to nickel and in normal human subjects*

A complex of NiCl₂ and bovine albumin 1:50 was used as antigen, corresponding to a concentration of bovine albumin of 15 mg/ml

Patients with positive patch test to nickel (migration index)	Controls (migration index)
0.31	0.51
0.45	0.75
0.33	0.71
0.61	0.55
0.57	0.73
0.45	0.61
0.48	1.04
0.68	0.67
0.49	0.71
0.56	
Mean 0.49	0.70
S.E.M. ± 0.014	0.023

$p < 0.01$



(Wilcoxon's two sample test)

Fig. 1. Leukocyte migration indices from patients with contact allergy to nickel and from normal human subjects. Complexes of NiCl_2 and bovine albumin 1:50, human albumin 1:125 and human epidermis protein 1:2 were

used as antigens. The concentrations of proteins were 3.5 mg human albumin/ml and 0.05 mg epidermis protein/ml.

tients was 0.64 and 0.84 for controls. The differences are significant ($p < 0.01$ and 0.02). In all cases an overlap was found between the two groups.

DISCUSSION

Contact allergy to nickel was demonstrated by the LMT using nickel protein complexes. No specific inhibition was found when NiCl_2 was used without conjugated protein. Specific transformation to nickel has been shown in lymphocyte transformation tests (7, 8, 10, 11). Nickel was used in the form of NiCl_2 (11), NiSO_4 (7, 8, 10) and nickel acetate (12). An inhibitory effect on the PHA-induced mitose activity in leukocytes has been found at higher nickel concentrations (1). Gimenez-Camarasa also showed an inhibitory effect with high concentrations of NiSO_4 (7). The concentration of Ni^{++} in the present study was approximately 90 $\mu\text{g}/\text{ml}$ in the culture chambers, i.e., within the

range of concentrations used in the LTT (7, 11). Bovine albumin alone gave no specific inhibition of leukocyte migration in patients with contact allergy to nickel; in both patients and controls an equal stimulatory effect could be seen.

The specific inhibition of peripheral leukocytes demonstrated in all nickel protein complexes indicates that more than one type of protein may be used as carrier protein in the test. It is noteworthy that nickel alone gives no specific inhibition in the LMT. One explanation for the finding of specific inhibition in LTT with nickel salts and without proteins may be that the long culture period could allow Ni^{++} to bind soluble proteins in the culture medium. The necessity of a carrier protein in LMT has earlier been demonstrated in patients with contact allergy to chromate (16). The apparent lack of carrier specificity in the present study does not necessarily need to be a true lack of specificity. Similar findings have been demonstrated in

chromium hypersensitivity (16). These patients showed specific inhibition to various chromium protein complexes, while patients with contact allergy to other substances, among these nickel, demonstrated no inhibition in LMT to chromium protein complexes. These results as well as the present findings could simply be a manifestation of delayed hypersensitivity against several metal protein compounds. Probably a number of conjugates are formed in the skin upon contact with nickel. In epidermis, several soluble proteins have been isolated, among them the same as are present in serum (2). In guinea pigs it has been demonstrated that sensitization with a hapten leads to formation of a set of protein conjugates to which the animal may react (13), and cross reaction between bovine albumin and human albumin has earlier been suggested by Gell & Benacerraf (6).

A complicating factor in the *in vitro* testing with nickel protein complexes is that these complexes are not particularly stable and that the quantity of bound nickel depends on the concentration of free nickel ions in the solution (4). The overlap between results from patients and controls is a limiting factor for the practical use of the LMT in a normal test situation. It seems that at present the test with nickel as antigen should only be used when studying groups of patients.

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