

## In vitro Studies on Blastogenic Lymphokine Activity in Nickel Allergy

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**Abstract.** By using gravity sedimentation separation, lymphocytes from some nickel-sensitive subjects can be made to produce blastogenic lymphokine when cultured with 14  $\mu\text{g NiSO}_4 \cdot 7\text{H}_2\text{O/ml}$ , as evidenced by the transforming effect of supernatant on control cells.

**Key words:** Lymphocyte transformation; Nickel allergy; Blastogenic lymphokine

In the human, sensitizing chemicals, e.g. dinitrochlorobenzene (DNCB), when applied epicutaneously, cause allergic contact dermatitis in previously sensitized individuals. This is a lymphocyte-mediated immune response of the delayed type and much of dermatological practice devolves on evaluating the role of sensitizing chemicals in the aetiology of a patient's dermatitis. Nickel sensitivity is common. Good correlation can be obtained between patch testing *in vivo* and blastogenic response *in vitro* to appropriate concentrations of nickel salts in lymphocyte cultures (2, 5). It is not clear at what point in the afferent limb of the immune response hapten protein conjugation occurs. Although it complexes with albumin and various amino acids (4), nickel can also bind directly to the lymphocyte cell membrane *in vitro*, as shown by autoradiography (3, 6). *In vivo*, Langerhans' cells probably 'process' the antigen before passing it on to lymphocytes. Recruitment of uncommitted lymphocytes by lymphokines elaborated by dividing cells of the sensitized clone, may be responsible for the spread of clinical dermatitis.

In this paper, we describe experiments designed to demonstrate whether production of blastogenic lymphokine by stimulated nickel-sensitized lymphocytes occurs *in vitro* and is capable of transforming lymphocytes from control subjects.

### MATERIALS AND METHODS

Heparinized blood (10 units/ml, bacteriostat-free) from 6 subjects with clinical evidence of contact dermatitis due to

Table I. Lymphokine activity of nickel-stimulated cultures

Subjects (paired nickel subject and control)	TUR			
	B/A	D/C	F/E	H/G
1	5.4	1.1	2.3	0.8
2	1.8	1.0	1.4	0.8
3	3.7	0.8	0.6	2.2
4	1.6	3.9	0.5	1.1
5	11.0	5.0	1.0	3.0
6	6.5	0.9	4.0	6.0
Mean	5.0	2.1	1.6	2.3
TUR >2	4/6	2/6	2/6	3/6

nickel, confirmed by patch testing, and from paired controls, was sedimented under gravity for 2 hours, the leukocyte-rich supernatant (>65% lymphocytes) centrifuged at 150 g for 15 min and the cell pellet washed three times with Medium 199 supplemented with L-glutamine and HEPES buffer and the cells re-suspended at a concentration of  $2 \times 10^6$  per ml in this medium containing 20% autologous plasma. Test tubes (100  $\times$  12 mm) with silicone bungs, containing 1.8 ml aliquots were set up in quadruplicate with nickel sulphate 14  $\mu\text{g per ml}$  ( $\approx 10^{-4}$  mEq Ni/ml) and without nickel (i.e. the basic LTT, tubes A-D in diagram 1). These were incubated at 37°C for 6 days. On day six, 4 hours prior to harvest, 12  $\mu\text{Ci}$  tritiated thymidine ( $^3\text{H}$ ]Td) was added to each tube. At harvest the cells were washed, dried and thymidine uptake measured using a liquid scintillator. The thymidine uptake ratios (TUR) of stimulated to control cultures were calculated.

A duplicate set of cell cultures A-D were centrifuged at 600 g for 10 min on day 3 and 0.9 ml of the supernatant was transferred in a cross-over pattern as shown in diagram 1 to 0.9 ml aliquots of cell suspension prepared as previously described, using fresh samples of venous blood (drawn on day 3 from the subjects) but without further addition of nickel (tubes E-H).

### RESULTS

Blastogenic lymphokine activity in the supernatant was detected in subjects 3, 5 and 6 as evidenced by increased TURs of 2.2, 3.0 and 6.0 respectively (Table I).

### DISCUSSION

14  $\mu\text{g NiSO}_4 \cdot 7\text{H}_2\text{O/ml}$  ( $\approx 10^{-4}$  mEqNi/ml) of culture is the optimal concentration to induce maximum thymidine uptake in the lymphocyte transformation test (LTT) in nickel-sensitive subjects and TURs >2 correlate closely with clinical and patch test evidence of nickel sensitivity (2, 5).

In a preliminary series of microwell cultures using this optimal concentration of nickel, predictably higher TURs were obtained with gravity-

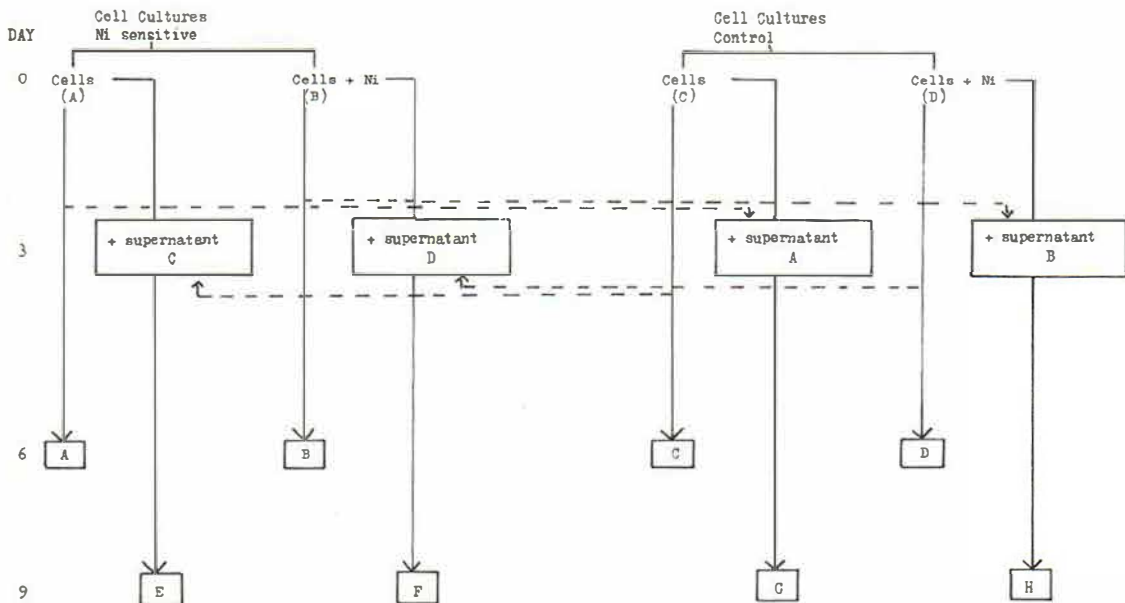


Diagram 1. Blastogenic lymphokine activity—culture protocol.

sedimented cells than with Ficoll-Paque-separated cells. Although Ficoll-Paque separation is the standard technique designed to give the highest yield of lymphocytes (1) it may be that helper cells important in the immune response are removed from the resulting purified cell population.

The method employed to demonstrate lymphokine activity was devised to ensure that if non-specific stimulatory or suppressive factors were elaborated *in vitro*, they would be detected. Evidence of blastogenic lymphokine production has been demonstrated. Further confirmation would require culture on a larger scale and separation of the factor by dialysis and/or chromatographic methods, since the nickel response *in vitro* is weak by comparison with some other antigens.

That blastogenic lymphokine activity can be demonstrated *in vitro* suggests that release of lymphokines *in vivo* may occur similarly with further antigen challenge in nickel-sensitized individuals.

Such an event would lead to the rapid recruitment of uncommitted lymphocytes and explain the "flare" phenomenon seen clinically on subsequent exposure to nickel, either locally or by ingestion.

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