In vitro Nickel Binding to Mononuclear Cells in Peripheral Blood

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Abstract. Mononuclear cells from the peripheral blood of four nickel-sensitive persons and 4 controls bound equal amounts of ^{63}nickel in vitro. 90–99% (mean 96%) of the ^{63}nickel was removed from the cells by washing three times every 30 minutes for 120 minutes. For 4 other patients and 4 controls, lymphocyte transformation with autologous nickel-incubated, irradiated cells as the stimulant showed that nickel was bound to the cells in sufficient quantities and was present in a form which could induce lymphocyte transformation in vitro in nickel-sensitive patients.

Key words: Lymphocyte transformation; Mononuclear cells; ^{63}Nickel; Nickel binding; Nickel hypersensitivity

Inorganic nickel salts have been used successfully both in the lymphocyte transofrmation test and the leukocyte migration inhibition test to demonstrate nickel hypersensitivity in vitro (2, 4, 7). In such experiments it has been assumed that the hapten binds to protein or cells in the culture medium, to become a complete antigen.

It has been shown morphologically in an autoradiographical study that ^{63}nickel binds to lymphocytes (3).

The following study was conducted to investigate the stability of the in vitro binding of nickel to mononuclear peripheral blood cells from nickel-sensitive patients and healthy individuals and to determine whether nickel coupled to mononuclear cells could induce lymphocyte transformation in vitro of autologous lymphocytes from nickel-sensitive patients.

MATERIALS

Eight patients who demonstrated at least infiltration and papules 72 hours after patch testing with 5% nickel sulphate in petrolatum and 8 controls participated in the study. Venous blood was drawn in equal amounts of RPMI-1640 medium (Gibco) with 20 I.U. of heparin per ml, and the mononuclear cells were separated by centrifugation (500 *g*) after layering on lymphoprep (Nyco). In order to reduce the quantity of serum proteins bound to the cells, these were washed three times in RPMI-1640 medium.

^{63}Nickel: Nickel chloride in 0.1 M hydrochloric acid with 0.97 mCi ^{63}Ni per ml was used (86 μg Ni/ml) (The Radiochemical Centre, Amersham, England). From this stock solution a 50-fold dilution was made with saline.

METHODS

^{63}Nickel study

For 4 of the patients and 4 controls 50 μl ^{63}Ni dilution (approximately 1 μCi ^{63}Ni) was added to 10^5 mononuclear cells in 0.25 ml of RPMI-1640 medium. Triplicates of four identical cultures were made from the cells of each person. The cultures were incubated at 37°C in a humid atmosphere to which 5% CO_2 was added. After 30 min of incubation three culture tubes of cells from each person were washed three times in RPMI-1640 medium and then harvested on glass filter paper with a Skatron® semi-automatic harvester. The remainder of the cells were washed three times and re-incubated. At 30 min intervals during a culture period of 120 min three tubes of cells from each person were washed and harvested as described above. Radioactivity was measured by scintillation counting. A blank and 4.8 μCi ^{63}Ni were used as standards in the scintillation counter, and the radioactivity was expressed as counts per minute (medians of the triplicate cultures). In initial experiments both the radioactivity of the cells and the supernatant were determined, and summation of the counts showed that at least 90% of the ^{63}Ni was recovered. Cell counts were performed on cultures incubated and washed as described above, and the loss of cells after washing three times was 5% or less.

Lymphocyte transformation study

Throughout this part of the study 15% pooled human serum and 480 000 units of penicillin, 0.5 g streptomycin, 50 mmol HEPES, 1.2 mmol glutamine and 20 000 units of heparin were added to each litre of RPMI-1640 medium.

One-third of the cells from the remaining 4 patients and 4 controls were incubated with 0.1% $NiSo_4$, $7H_2O$ (Specpure®) as described above, while two-thirds of the cells were incubated without nickel.

After 30 min of incubation 10% of the nickel-conjugated cells and half of the non-nickel-conjugated cells were subjected to 2 400 rad gamma irradiation in order to arrest multiplication without destroying the cells. Following the irradiation the cells were washed three times with RPMI-1640 medium.

Cultures of 10^5 cells were made in 0.5 ml medium. The following stimulants were used in triplicate cultures from each person: (1) nickel sulphate, 50 μl of a 1 : 100 dilution of a 1% stock solution; (2) nickel sulphate, 50 μl of a 1 : 200 dilution of a 1% stock solution; (3) 10^5 irradiated, nickel-conjugated autologous cells; (4) 10^6 irradiated nickel-conjugated autologous cells; (5) 10^5 irradiated non-nickel-conjugated autologous cells; and (6) 10^6 irradiated non-nickel-conjugated autologous cells. Triplicate unstimulated cultures served as controls. Identical cultures were incubated for 120 and 144 hours. ^{14}C-labelled

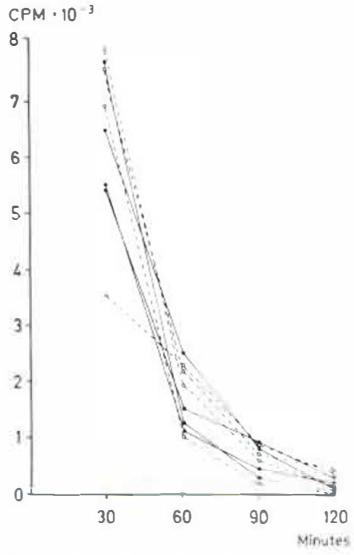

Fig. 1. Binding of ^{63}Ni to mononuclear cells. Counts per minute (cpm) given as median of triplicates for each patient (●) and control (○) after 30 min of incubation and following three washings every 30 min for 120 min.

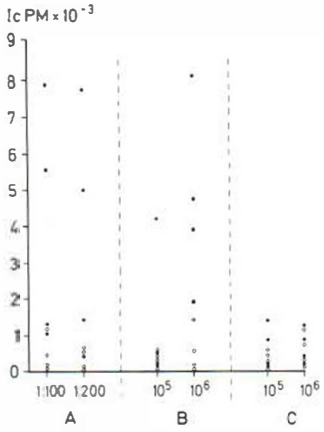

Fig. 2. Nickel-induced lymphocyte transformation. Results of lymphocyte transformation test using as the stimulators (*A*) 1:100 and 1:200 dilutions of nickel sulphate, (*B*) 10^5 and 10^6 irradiated nickel-conjugated autologous cells, and (*C*) 10^5 and 10^6 irradiated non-nickel-conjugated autologous cells. Patients = ●, Controls = ○. Results are given in increment counts per minute (icpm). (For explanation, see text.)

thymidine was added to the cultures for the last 24 hours. The cultures were harvested on glass filter paper, and liquid scintillation counting was performed in Instagel® (7). The results were expressed as increment counts per minute, i.e. counts per minute of stimulated cultures (median of triplicates) minus counts per minute of unstimulated cultures (median of triplicates).

RESULTS

^{63}Nickel bound to the mononuclear cells after an incubation period of 30 minutes. There was equal binding to the cells from both patients and controls (Fig. 1). The binding was reversible, and after washing three times in RPMI-1640 medium at 30-min intervals for 120 min 90–99 % (mean 96 %) of the ^{63}nickel was removed from the cells.

The results of the lymphocyte transformation study after culture periods of 120 and 144 hours were similar. The results after a 120-hour culture period are depicted in Fig. 2. After stimulation with nickel sulphate, differentiation could be seen between patients and controls. The use of 10^5 irradiated nickel-conjugated autologous cells produced stimulation only in the patient who had the highest counts when nickel was present in the medium. After stimulation with 10^6 nickel-conjugated autologous cells, distinct differentiation between patients and controls was seen ($p<0.025$, Mann-Whitney test). Irradiated non-nickel-conjugated autologous cells produced no stimulation.

DISCUSSION

Since equal binding took place in cell suspensions from nickel-hypersensitive patients and controls, the above results indicate that the great majority of the nickel binds non-specifically to mononuclear cells of the peripheral blood. This binding is reversible, and 90 % or more of the nickel can be removed by washing the cells every 30 min for 120 min. That the nickel bound to irradiated autologous mononuclear cells induced specific lymphocyte transformation also indicates reversibility of the binding of nickel to the mononuclear cells.

Nickel levels in serum do not vary greatly (6). The capacity of mononuclear cells to bind nickel reversibly may in part explain the widespread flares of nickel dermatitis sometimes seen in nickel-sensitive persons after ingestion of nickel (1, 5).

Nickel binds strongly to albumin (6), and it could be assumed that the ^{63}nickel bound to albumin adheres to the mononuclear cells. However, the cells were washed six times before the initial determination of radioactivity, and accordingly, this explanation for the binding of nickel would seem unlikely. The rapid decline of the ^{63}nickel concentra-

tion after further washing also indicates that the nickel was, in fact, bound to the cells.

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Cell-mediated Immune Response to Basal Cell Carcinoma

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Abstract. The role of cell-mediated immunity in controlling Basal Cell Carcinoma (BCC) growth was studied by measuring the transformation of lymphocytes when challenged *in vitro* with aqueous extracts of the patients' BCCs and of control skin. Tests were carried out in the presence of autologous and heat-treated plasma. Three patients out of 8 showed significantly raised thymidine uptake ratio (TUR). Heat-treated plasma produced higher TURs, indicating the presence of an inhibitory factor.

Key words: Basal cell carcinoma: Aqueous tumour extract: Lymphocyte transformation: Cell-mediated immunity

Tumors may develop as a result of exposure to carcinogens (chemical, physical or viral), defects in DNA repair, and deficiency of immunological surveillance. The common Basal Cell Carcinoma (BCC) of the skin is slow growing, has limited invasive properties, and is only locally malignant. Histologically the tumour is associated with a variable degree of lymphocytic infiltrate. Does the tumour grow autonomously in an "indifferent" environment or is there an active contest between host and tumour, involving a cell-mediated immune response? We report here experiments involving challenge of patient's lymphocytes with extracts of their BCCs and assessment of the resulting blast transformation.

MATERIALS AND METHODS

BCCs were excised under local anaesthetic (1% lignocaine) from 8 patients. At the same time a control biopsy was obtained (with informed consent) from a skin site remote from the tumour (upper outer arm). Histological confirmation of the diagnosis was obtained from a portion of the tumour. A portion of the remainder (100–400 mg) was weighed, minced, homogenized in 2 ml physiological saline using a tissue disintegrator, and the extract filtered through a 0.45 μm Millex filter. The normal skin sample was treated similarly.

20 ml heparinized venous blood was obtained from each patient and was separated under gravity for 1–2 hours. The white cell layer was removed and total and differential cell counts carried out which confirmed a lymphocyte content of 50–70%. The plasma was separated from the remaining cells by centrifugation at 150 *g* for 10 min. Half the plasma was heated at 56°C for 30 min to destroy complement. Cells were washed in Medium 199 (Wellcome) and re-suspended in Medium 199 to contain 2×10^6 cells per ml.

110-μl aliquots of this cell suspension were placed in microwells, supplemented with 30 μl plasma and 10 μl of the following constituents: (*a*) physiological saline (as baseline control); (*b*) BCC extract (BCC 1); (*c*) BCC extract, 1 in 10 dilution in physiological saline (BCC 2); (*d*) control skin extract (Cont. 1); (*e*) control skin extract, 1 in 10 dilution in physiological saline (Cont. 2). All samples were set up in quadruplicate. The series was duplicated using the heat-treated plasma instead of the normal plasma.

After incubation at 37°C for 6 days, 2 μCi [^{3}H]thymidine (RCC Amersham) in 10 μl Medium 199 was added to each