

Human Leukocyte Cyclic AMP and Cyclic GMP Levels during Chemotaxis in Delayed Type Hypersensitivity

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Ten nickel-allergic patients and six healthy control subjects participated in a study of the morphology, kinetics and evolution of the cAMP and cGMP concentrations of migrated leukocytes, using an improved skin chamber technique. Also studied was the effect of nickel exposure in the chamber medium during development of an eczematous reaction in the nickel-allergic patients. Nickel exposure had a specific effect on the morphology, from the 24th hour to the end of the 48 h observation period, with a significant increase in the percentages of basophils, eosinophils and lymphocytes and a decrease of neutrophils. A significantly increased leukocyte migration rate (LMR) was observed from the 27th to 39th hour in six of the allergic patients exposed to nickel. There were no specific permanent changes in cAMP and cGMP concentrations during nickel exposure. The control chambers of the allergic patients and healthy controls had identical leukocyte morphology, LMR and leukocyte concentrations of cAMP and cGMP. However, no correlations were found between LMR, cAMP and cGMP in the eczema patients throughout the observation period.

Key words: cAMP and cGMP; leukocyte chemotaxis; nickel allergy.

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Chemotaxis and chemokinesis are considered the most important processes for the induction of leukocyte migration to sites of inflammation. Chemotaxis is the directed locomotion of cells, induced by a gradient of chemical substance. Chemokinesis is the non-directed locomotion. Both chemokinesis and chemotaxis are preceded by the binding of chemoattractants to specific receptors on polymorphonuclears (PMN) and monocytes (25). The molecular events that occur within a few minutes of this binding are not fully known, but some components are well described. Changes in membrane potential are followed by other ionic events (4), increase of cyclic AMP (14, 19, 20), alteration in phospholipid methylation (10, 11) and activation of arachidonate metabolism (11, 25). These events are followed by reorganization of cytoskeletal elements, including a change in

shape (polarization) (4). During the induction phase of chemotaxis and chemokinesis, measurements of PMN cAMP have shown an increased concentration. Also, sustained changes of PMN cyclic nucleotide levels seem to modulate chemotaxis. Agents that elevate cAMP, such as B-adrenergic agonist, phosphodiesterase inhibitors and histamine (7, 21), depress chemotaxis, whereas agents that elevate cGMP enhance chemotaxis (9, 23, 24). The mechanism by which intracellular cyclic nucleotide changes effect leukocyte locomotion is unknown, although they are suggested to affect microtubule assembly either directly or through an effect on stored Ca^{2+} (1, 16). To elucidate the proposed importance of cyclic nucleotides in the regulation of chemotaxis, release of enzymes (26, 27, 32) and phagocytosis (8, 12, 18), we have measured the *in vivo* cell migration

and the cyclic nucleotide concentrations of mobilized leukocytes. We found it of special interest to investigate nickel-allergic patients during the development of an eczematous reaction, as a model of a cell-mediated immune reaction. Allergen-activated lymphocytes release a number of soluble lymphokines *in vitro* (22). The morphology and migration of exudated leukocytes in nickel allergy (15) may be explained by lymphokine activities. A recent study by Bendtzen et al. (2) indicates that changes in cyclic nucleotides modulate the effect of lymphokines. It is unknown whether the cell-mediated immune reaction produces permanent changes in the cyclic nucleotide levels of the activated leukocytes. By use of the skin chamber technique with nickel exposure we have attempted to answer this question.

METHODS

Subjects

The study comprised 16 volunteers of both sexes, divided into two groups.

Group I consisted of 10 patients (Nos. 1–10) (8 females, 2 males); mean age 37 years (age range 20–65), all had an allergic contact dermatitis to nickel, clinically verified by the history and patch tests. Patch testing was performed according to the recommendations of the International Contact Dermatitis Research Group with 5.0% nickel sulphate in petrolatum. In all cases the nickel patch tests were strongly positive (erythema, oedema and vesicles). At the time of the investigation the eczema was inactive or only mildly active. The forearm was uninvolved in each patient, and the eczema never exceeded 5% of the skin surface.

Group II consisted of six healthy controls (Nos. 11–16) (5 females and 1 male); mean age 26 years (age range 23–32), with no eczema or history of allergic contact dermatitis. Patch tests for nickel sulphate, potassium dichromate and cobalt chloride were negative in these patients.

Neither group was undergoing medical treatment at the time of the investigation and no

systemic glucocorticoids had been given for at least 6 months, and no other antiinflammatory drugs (including topical glucocorticoids) for at least 3 days. Patient No. 9 had psoriasis and patient No. 14 suffered from seborrhoeic dermatitis and multiple sclerosis. None of the patients had any signs or history of atopic disease. All patients showed normal blood leukocyte and differential counts.

Informed consent, in accordance with the principles of the Helsinki Declaration was obtained from all subjects.

Skin chamber technique

A dermabrasion was performed on the inner surface of both forearms of the patients with nickel hypersensitivity and on the right forearm of the controls, as previously described (15). Briefly, an abrasion of the epidermis was performed with a surgical scalpel leaving a uniformly glossy area with the capillary network of the corium visible but no bleeding. The denuded area varied in size between 1.5 and 6 cm². A sterile chamber of epoxy material was sealed over the skin window with glue (Ducocement Dupont de Nemours SA, Belgium) and hypoallergic tape.

In order to evaluate the surgical removal of epidermis, biopsies were taken from different areas of the skin windows. The histology of the dermabrasion revealed almost total removal of the epidermis leaving the basal membrane and one or more layers of stratum spinosum intact.

Chamber media and collection procedure

The chamber medium was autologous serum obtained 24 h prior to the investigation under strictly sterile conditions and left at 4°C to allow complete degradation of cyclic nucleosides in serum. The right chambers of the eczema patients and controls were filled with 0.5 ml autologous serum containing 10 mM EDTA immediately after the chambers were applied and at each collection time. EDTA was added to the media to prevent degradation of cyclic nucleotides from the exudate of the skin window. The chamber medium used in the left chamber of

the nickel-allergic patients was 0.5 ml autologous serum with 10 mM EDTA and 10 mM nickel sulphate. Chamber medium was collected every 3rd hour during the daytime and evenings from all patients. Supplementary collections were performed during the night periods. Collected cells were counted in a modified Neubaur haemocytometer. Smears stained by the May-Grünwald-Giemsa technique were done on aliquots of the collected cells, and the frequency of the different leukocytes was calculated in a count of 500 cells. The numbers of mobilized cells migrated per hour per cm² of denuded areas were computed.

cAMP and cGMP determinations

Immediately after the collected cells had been separated by centrifugation, the enzymatic activity of the cells was terminated by boiling for 3 sec (6). Radioimmunoassay was used for deproteinization of the cells with ethanol and quantitation of cAMP and cGMP, as previously described (16, 29).

Statistics

The morphology of the migrated leukocytes was evaluated by Wilcoxon's rank sum test. The

variation in time of the leukocyte migration rate (LMR) and the concentration of cAMP and cGMP were evaluated by two-way analyses of variance of the logarithmic observations. The differences between LMR, cAMP and cGMP measurements in the right and left arm of the eczema patients were appraised by paired Student *t* tests (logarithmic scale) (using a pooled variance for all patients).

RESULTS

Clinical response and morphology of the chamber cells

The clinical and morphological response to nickel exposure has been previously published (15). The numbers of eosinophils and basophils were sparse, and as we were aware that differential counting of only 100 cells might be insufficient for a detailed analysis, 500 cells were counted in this study to enable proper statistical analyses. The data obtained are presented in Table 1. A significant increase in the percentages of eosinophils and basophils in the nickel-exposed chambers was observed from the 24th hour to the end of the observation period, followed by an increase of lymphocytes and a decrease of neutrophils.

Table 1
Cellular morphology in the exudate at 12, 24 and 42 h after start of skin window

	PMN (%)	Lymphocytes (%)	Monocytes (%)	Basophils (%)	Eosinophils (%)
12 hours					
Healthy volunteers	98.1 (95.3-99.2)	1.6 (0.8-4.4)	0 (0-0.3)	0 (0-0.6)	0 (0-0)
Nickel-allergies without nickel	97.2 (95.4-99.4)	1.3 (0-3.6)	0.4 (0-3.2)	0.2 (0-0.6)	0 (0-0.2)
Nickel-allergies with nickel	98.0 (91.0-99.2)	1.3 (0.4-4.5)	0.4 (0-2.5)	0.2 (0-2.0)	0 (0-0)
24 hours					
Healthy volunteers	94.4 (89.6-98.6)	3.8 (1.0-5.4)	0.2 (0-1.8)	0.2 (0-0.8)	0 (0-0.2)
Nickel-allergies without nickel	97.3 (87.6-98.6)	1.7 (0.4-11.4)	0.6 (0-1.4)	0.1 (0-1.8)	0 (0-0.2)
Nickel-allergies with nickel	94.0 (85.2-97.2)	3.0 (0.8-9.0)	0.6 (0.2-1.6)	1.5 (0.4-3.0)*	0.3 (0-6.6)*
42 hours					
Healthy volunteers	97.4 (97.0-99.8)	1.8 (0.2-2.4)	0.2 (0-0.6)	0.2 (0-0.4)	0 (0-0)
Nickel-allergies without nickel	96.7 (92.8-99.6)	1.6 (0.2-6.0)	1.0 (0-2.3)	0.2 (0-1.6)	0 (0-0)
Nickel-allergies with nickel	86.2 (73.8-97.8)*	7.1 (0-21.6)*	0.8 (0.4-1.6)	2.3 (1.6-6.8)*	0.3 (0-6.8)*

Median value and observed range by the counting of 500 cells at each collection. Differences between groups are indicated by **P* < 0.05 (Wilcoxon's rank sum test).

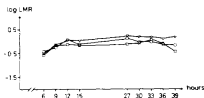


Fig. 1. Average profiles of the mean values (logarithmic scale) for leukocyte migration rates (LMRs) during the observation period for controls ($\square$ — $\square$), nickel-allergic patients not exposed to nickel ($\circ$ — $\circ$), and nickel-allergic patients exposed to nickel ($\triangle$ — $\triangle$).

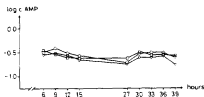


Fig. 2. Average profiles of the mean values (logarithmic scale) for cyclic AMP concentrations of migrated leukocytes during the observation period for controls ($\square$ — $\square$), nickel-allergic patients not exposed to nickel ($\circ$ — $\circ$), and nickel-allergic patients exposed to nickel ($\triangle$ — $\triangle$).

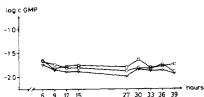


Fig. 3. Average profiles of the mean values (logarithmic scale) for cyclic GMP concentrations during the observation period for controls ($\square$ — $\square$), nickel-allergic patients not exposed to nickel ($\circ$ — $\circ$), and nickel-allergic patients exposed to nickel ($\triangle$ — $\triangle$).

Leukocyte migration rate (LMR)

The kinetics of leukocyte mobilisation are elucidated by the measurement of LMR. All patients showed a low, but increasing LMR during the first 6 h, during the rest of the

observation period the LMR fluctuated around a higher level. The profiles depict an almost identical pattern for all groups during the 6th to the 39th hour. An analysis of the congruency of the average profiles of LMR (Fig. 1) between each group in this period revealed no significant differences ($P > 0.05$).

Subsequently, the LMR levels in each eczema patient with and without nickel exposure were tested by paired Student's *t* test during the 6th to 15th hour and 27th to 39th hour. In the latter period six patients (Nos. 1, 2, 3, 4, 7, 8) had an increased LMR ($P < 0.05$) in the nickel-exposed chamber. Three patients (Nos. 6, 9, 10) showed no change in LMR and one patient (No. 5) had a statistically higher LMR ($P < 0.05$) in the chamber without nickel exposure.

The addition of 10 mM EDTA to the autologous serum revealed no difference in LMR (data not shown).

cAMP and cGMP concentrations in the migrated leukocytes

At the 3 h and 6 h collections the concentrations of the two nucleotides were high, but during the remainder of the observation period only small oscillations were observed. cAMP concentrations were about 260 fmol/ 10^6 leukocytes and cGMP concentrations about 17 fmol/ 10^6 leukocytes, and both nucleotides exhibited characteristic diurnal fluctuations from the 6th to the 39th hour. cAMP and cGMP profiles of both groups showed almost identical oscillation patterns between the 6th and the 39th hour.

An analysis of congruency of the average profiles for cAMP (Fig. 2) and cGMP (Fig. 3) revealed no significant differences between the groups ($P > 0.05$).

To examine whether nickel exposure changed the cAMP and cGMP levels in the chambers of the allergic patients, the cAMP concentrations in the chambers with and without nickel exposure were compared. The results showed no difference in any patients, except for patient No. 4, who had a statistically lower cAMP concentration ($P < 0.05$) in the nickel-exposed chamber throughout the entire ob-

servation period. The corresponding results for cGMP revealed that four patients (Nos. 2, 5, 6, 7) had a low cGMP level in the exposed chamber from the 6th to the 15th hour and four patients (Nos. 1, 2, 4, 5) from the 27th to the 39th hour.

Relation between LMR, cAMP and cGMP in migrated leukocytes

The results in Figs. 1, 2 and 3 indicate a reverse relation between LMR and the two cyclic nucleotides. However, no correlations were found between LMR, cAMP and cGMP in an analysis of the difference between the chamber values for the eczema patients through the observation period.

DISCUSSION

The morphological picture of the skin window exudate during nickel exposure is qualitatively consistent with the morphological picture obtained by skin biopsies from allergic contact dermatitis patients (3, 5), except that mononuclear cells dominate in skin biopsies while PMN dominates in the exudate. This discrepancy may be ascribed to the character of the allergen-exposed skin window, being a combination of a specific and non-specific inflammation. In agreement with other studies (3, 13, 31) of allergic contact dermatitis using different techniques, the early appearance of basophil leukocytes, approx. 24 h after the initial application of an allergen, seems to be a characteristic finding in allergic hypersensitivity reactions.

Leukocyte migration is the result of humoral and cellular factors and depicts the mobilisation to an inflammatory lesion via the blood (30). The LMRs of the eczema patients with nickel exposure exhibited a different mobilisation pattern from the 27th to the 39th h after dermabrasion. The difference in LMR between the two chambers had to be 55% or more to be statistically significant (on 5% level). Six patients had an increased LMR during this period, three remained unchanged and one had a reduced LMR compared with their own control chamber. The different mobilisation

pattern could not be related to the clinical response (15) or to the morphology of the exudate, thus humoral and cellular factors are the most likely explanation for this divergence. The alteration of cyclic nucleotide levels in the migrated leukocytes of eczema patients with and without nickel exposure did not correspond to the observed differences in LMR.

To our knowledge cAMP and cGMP in activated leukocytes during a hypersensitive reaction have not been previously studied. The initial high levels of both nucleotides at the first collections after dermabrasion may be ascribed to the release of inflammatory factors after the unspecific trauma. The concentrations of prostaglandins in the skin window exudate are high during the first hours of dermabrasion (data not shown), and may have influenced the high nucleotide concentrations.

During the remainder of the observation period the concentrations of both nucleotides stabilized at lower levels, with a characteristic diurnal rhythm identical to that of the controls. The exposure of nickel revealed no permanent specific changes in nucleotide levels. Our technique does not rule out the possibility that short-term (within seconds and minutes) nucleotide changes had occurred, as observed during chemotaxis (14, 19), enzyme release (8, 27) and phagocytosis (8).

The difference in cAMP or cGMP concentrations between chambers of the allergic patients had to be at least 50 and 37%, respectively, to be statistically significant (on 5% level). Some of the allergic patients showed significant differences in cyclic nucleotide concentrations between chambers. These observations were not related to any other parameter observed in the study.

A study of the variation in time of the average profiles of LMR, cAMP and cGMP in healthy volunteers and nickel-exposed and non-exposed nickel-allergic patients revealed a tendency for LMR to increase when cAMP and cGMP decrease, and vice versa. The observed possible relation between LMR, cAMP and cGMP may be true, but it could also be that the determination of all three variables was based on cell counts, and therefore are not statistically inde-

pendent observations. Further investigations of this problem must await the introduction of other indicators of cyclic nucleotide concentration than cell counts. Preliminary data indicate that the release of some granulocyte proteins from the migrated cells could be used for this purpose.

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