In vitro Migration of Human Mononuclear Cells towards a Psoriatic Scale Extract

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Extracts of psoriatic scales from patients with chronic plaque psoriasis and of normal human post-mortem skin were tested for their effects on the migration of healthy human mononuclear cells in a modified Boyden chamber. A preparation of cells containing 12-32% monocytes was obtained from whole blood by a density gradient technique. The cells were allowed to migrate through a polycarbonate filter towards various dilutions of the two types of extract, a known chemoattractant or towards buffer alone. There was enhancement of migration towards psoriasis scale extract, with significant differences only at the stronger concentrations tested. The results suggest that scales taken from stable plaque psoriasis contain an extractable water-soluble factor which stimulates the movement of peripheral blood mononuclear cells. Key words: Chemotaxis; Monocytes. (Received May 7, 1984.)

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The inflammatory cell infiltrate in the upper dermis of a developing psoriatic plaque consists initially of macrophages and T lymphocytes (1, 2), and only later is the predominant cell the polymorphonuclear leukocyte. Investigation into the role of chemotactic factors in causing and maintaining this cellular infiltrate has focused mainly on the neutrophil, although there are two reports of the migration of mononuclear cells towards a psoriatic scale extract (3, 4).

The purpose of this study was to prepare an extract from the scales of patients with mild to moderate stable plaque psoriasis, and to compare the migration of mononuclear cells towards it, a normal skin extract and the known synthetic chemoattractant, N-formylmethionyl-leucyl-phenylalanine (FMLP).
MATERIALS AND METHODS

Mononuclear cells were obtained from the blood of healthy human volunteers by a density gradient technique (5), and resuspended in Gey's I medium (6) at a concentration of 3×10^6 cells/ml. In this preparation, the proportion of monocytes ranged from 12% to 32% as estimated either on morphological criteria or using an indirect immunofluorescent technique. Viability, as assessed by the trypan blue exclusion test, was greater than 96% in all but one test, when the value was 93%.

Aqueous psoriatic scale extract was prepared as previously described (7) from scales collected from untreated lesions of 13 patients with chronic plaque psoriasis who were free of systemic treatment for the skin condition. Control stratum corneum was obtained from glabrous skin of 10 cadavers without any known widespread skin disease within 48 hours of death. The method of preparation was altered slightly for the skin extract, such that the ratio of weight:volume used was 1:20. Then, by allowing a small aliquot to evaporate for approximately 36 hours at 37°C, the volume was reduced by half.

The migration of monocytes through a polycarbonate filter with a pore size of 5 µm (Nucleopore Corporation) was assessed in modified Boyden chambers. 0.2 ml of the cell suspension was placed above the filter in small hinged chambers, whilst below, there was approximately 90 µl of Gey's I buffer (for assessment of random unstimulated migration), various dilutions of the scale or skin extract, or FMLP (Backen Feinchemikalien AG, Bubendorf) in a concentration of 1×10^-7 M. The chambers were incubated at 37°C for 90 minutes, after which time, the filters were removed, fixed and stained. Some days later, the number of cells on the bottom surface of the filters were counted.

Seven volunteers provided blood for tests of migration towards psoriasis scale extract, and seven more for the tests with skin extract. Three people also provided blood twice, for tests with both types of extract.

The results were tested statistically using a two-factor analysis of variance.
RESULTS

The migration of healthy human mononuclear cells across a polycarbonate filter was greater towards the psoriatic scale extract than towards the cadaver skin extract (Fig. 1). A 2-factor analysis of variance was performed using (a) extract, i.e. the migration towards scale extract with that towards skin extract, and (b) treatment, i.e. the migration towards different dilutions of the same extract and FMLP. The analysis revealed an extract-treatment interaction \((F(4, 45)=2.571, p<0.05)\). There was a significant difference between mononuclear cell migration towards the different extracts only at full strength and 1:2 dilution. A significant dilution effect was present for both psoriasis scale and skin extracts, and further analysis according to the Newman–Keuls procedure revealed significant differences between the 1:8 dilution of scale extract compared to full strength solution, and the 1:2 dilution and FMLP. For the skin extract, migration towards FMLP was significantly different from migration towards the 1:2, 1:4 and 1:8 dilutions.

Three subjects gave blood twice so that the migration of their mononuclear cells could be measured towards both extracts. By applying the 2-factor analysis of variance to these results, the differences between migration towards the psoriatic scale and the skin extracts was significant only at full strength (Fig. 2). There was no significant dilution effect for the results for these three individuals.

DISCUSSION

These experiments demonstrate a marked increase in the locomotion of the mononuclear cells through a filter separating a suspension of cells from an extract of psoriatic scales. Previous experiments which have examined the migration of mononuclear cells towards psoriatic scale extracts (3, 4) have not used scales from stable plaques and did not take into account the possible effects that various treatments might have had on the chemoattractiveness of the scales. If substances found in psoriatic scales are of importance in initiating and then maintaining the plaque by some mechanism involving chemoattractant properties, then it is essential to look at scales taken from one particular stage or form of the disease, or during the course of a specified treatment.

Such simple extracts as used here obviously contain many water-soluble components, some of which could be chemoattractive or chemokinetic for human blood leukocytes. Extracts made from psoriatic scales collected at different stages during treatment may well alter in their chemotactic or chemokinetic effect on mononuclear cells, and this approach could further our understanding of what it is in a scale extract that stimulates their locomotion. The mere existence in psoriatic scales of factors stimulating migration of mononuclear cells might have therapeutic implications. Removal of the scales is a first step in most therapeutic models. This step might be valuable not only for enhancing the penetration of anti-psoriatic drugs but also for diminishing the infiltration of white blood cells into the psoriatic lesion.

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REFERENCES

Selenium, Glutathione-peroxidase and Dermatitis herpetiformis

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In patients with dermatitis herpetiformis, decreased blood levels of glutathione-peroxidase (GSH-Px) were common (1). GSH-Px is an index of selenium content and used to detect functional selenium deficiency. We report here the results of a double-blind study where selenium+vitamin E or placebo was added to the dapsone treatment in patients with dermatitis herpetiformis.

PATIENTS

Twenty-four patients (18 men and 6 women, age 32–75 years) were selected for the study. They had all a clinically classical dermatitis herpetiformis and granular deposits of IgA in the papillary tips. During the last year all had needed a constant dose of dapsone (25–200 mg daily) to be free from skin symptoms. For 12–72 months four of them had also used a gluten-free diet without being able to reduce their dapsone intake. Six patients on a normal diet had just started on a gluten-free diet. They were therefore excluded from the study, since it would be difficult to evaluate the clinical effect of selenium supplementation. The patients should be treated double-blind for 5 months twice daily either with placebo or tablets containing 0.2 mg of selenium (as Na2SeO3) + 10 mg tocopheryl succinate.

METHODS

Blood was drawn in 10 ml heparinized vacutainer tubes before treatment and after 2.5 and 5 months. GSH-Px was determined in hemolysed blood as described previously (1, 2). Selenium was in some patients also determined in erythrocytes and plasma by neutron activation analysis (3). The clinical effect of the given tablets was evaluated from the individual dapsone doses needed before and after the trial.

RESULTS

When the study began there was unfortunately a report in press and on television about carcinogenic effects of selenium. Three patients who had read or heard this report stopped