

lagenase. Only the use of desoxyribonuclease during the primary tryptic incubation inhibited the formation of this material and the cell clumps.

The autoradiographic analysis showed that the enzymatic dissociation of the epidermis with both trypsin and desoxyribonuclease did not affect DNA synthesis of the epidermal cells. On the contrary, the labelling index in the epidermal cell suspension was higher than the labelling index in the blister roof epidermis before the dissociation. There are many possible explanations for this phenomenon. The total cell count in the cell suspensions is lower than in the whole roofs, because some of the cells in the upper stratum malpighi remain attached to the keratin layer. Obviously, some epidermal cells are destroyed during the dissociation procedure (11). Furthermore, the diffusion of [³H]TdR may be better in the cell suspension than in the whole epidermis. Finally, there is great variation in the labelling indices between different blister roofs, clearly depending of the number of hair follicles and sweat ducts in the area.

According to previous studies the labelling index for normal human skin varies between 2.2 and 5.2% when calculated for the basal cell layer (7, 12). In order to compare the labelling index of the blister roofs with that of the cell suspensions we had to count the labelled cells for all epidermal cells. Therefore, the labelled cells in higher epidermal layers, presumably Langerhans' cells, are also included in our labelling indices (9). The proportion of labelled Langerhans cells in normal guinea pig epidermis is less than 10% of all labelled cells, and the total number of Langerhans cells in human skin is reported to be about the same as or less than in guinea pig skin (10). Therefore, by correlating our labelling index in blister roof epidermis to the number of epidermal layers in the abdominal skin area (about five) and to the supposed number of Langerhans cells our value is similar to the labelling index of normal human epidermis. Thus it seems that the suction procedure does not interfere with DNA synthesis in fresh suction blister roof epidermis.

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Polymorphonuclear Leukocyte Chemotaxis in Dermatitis Herpetiformis

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Abstract. Polymorphonuclear leukocyte chemotaxis was investigated in 15 patients with dermatitis herpetiformis and was found not to be significantly increased when compared with the polymorphonuclear leukocyte chemotaxis of 15 normal healthy controls. These results indicate that accumulations of polymorphonuclear leukocytes found in the skin of patients with dermatitis herpetiformis are not due to an increased responsiveness of polymorphonuclear leukocytes to a chemotactic factor.

Key words: Dermatitis herpetiformis; Chemotaxis

Dermatitis herpetiformis (DH) is an uncommon chronic blistering disease with distinct histopathological criteria and a variety of immunological abnormalities (7). The characteristic histological findings in early skin lesions are accumulations of polymorphonuclear leukocytes (PMN) which are distributed in the upper parts of the dermis, predominantly forming microabscesses in the tips of the dermal papillae (7).

The presence of inflammatory cells at a site of tissue injury is the consequence of chemotaxis, which designates the directed migration of cells towards a chemotactic factor gradient (3, 6). Increased or decreased responsiveness of cells to chemotactic factors has been described in several inflammatory or infectious skin diseases (3, 6, 11, 13). This study was performed in order to investigate whether PMN of patients with DH exhibit an increased responsiveness to a chemotactic factor.

MATERIALS AND METHODS

Patients

A total of 30 individuals were investigated, 15 patients with dermatitis herpetiformis (DH) (10 males and 5 females, mean age 46 ± 5 years), and 15 sex- and age-matched normal healthy volunteers. All DH patients exhibited the characteristic histopathological criteria and granular IgA deposits in the tips of the dermal papillae by immunofluorescence. (7)

At the time of investigation all patients had been undergoing sulfone treatment (mean daily dosage 68 ± 16 mg Avlosulfon®, Imperial Chemical Industries, Great Britain) for at least 6 months (mean duration of treatment 7 ± 1 years). Six patients were completely free of skin lesions, 9 patients showed relapses. None of the patients had clinical symptoms of gluten-sensitive enteropathy and none were restricted to gluten-free diet.

Chemotaxis assay

Polymorphonuclear leukocytes (PMN) were obtained by sedimentation of heparinized venous blood with 2% Dextran 500 (Pharmacia, Uppsala, Sweden) (14). Contaminating erythrocytes were lysed by 0.84% NH_4Cl . PMN were washed three times in medium RPMI 1640 (Flow Laboratories, Bonn, West Germany) containing 100 IU penicillin/ml, 100 μg streptomycin/ml (Gibco, Glasgow, Scotland), 2% bovine serum albumin (Fluka, Buchs, Switzerland) and were then adjusted in medium to a concentration of 2×10^6 cells/ml. Trypan blue exclusion revealed more than 94% viable cells in all experiments.

Chemotaxis was determined by a modification (5) of Boyden's micropore filter technique (1). Cell suspensions (0.5 ml) were placed in the upper compartment of the chemotaxis chamber. Pooled human serum (1.5 ml) activated by incubation with zymosan (Koch-Light Laboratories, Colnbrood, Bucks, UK) and diluted in gela-

tin-veronal-buffered saline (pH 7.3) according to Clark & Klebanoff (2) served as chemotaxin in the lower compartment. Filters 3 μm pore size (Millipore Corporation, Bedford, Mass., USA) were used. After the incubation for 120 min at 37°C, filters were detached, stained with haemalum and the cells present on the lower side of the filters were counted in 20 oil immersion fields. Assays were performed in triplicate and the results were expressed as chemotaxis index = mean number of cells/oil immersion field \pm standard error of the mean (S.E.M). The random mobility of PMN was evaluated by determining the chemotaxis index, using gelatin-veronal-buffered saline without the chemotaxin in the lower compartment of the chemotaxis chamber.

Statistical analysis was performed according to Student's *t*-test.

RESULTS

The mean chemotaxis index (\bar{x} CI) of PMN derived from 15 patients with DH was 3.2 ± 0.9 and was found to be not significantly different ($p > 0.2$) from the \bar{x} CI ($= 3.4 \pm 0.2$) of PMN from 15 normal healthy controls. No significant difference was observed in the CI of patients who were completely free of skin lesions or exhibited relapses of the disease. In addition no significant difference ($p > 0.2$) was observed in the random mobility of PMN of patients with DH (\bar{x} CI $= 0.6 \pm 0.1$) when compared with the controls (\bar{x} CI $= 0.7 \pm 0.1$). These results indicate that the responsiveness of PMN from patients with DH to zymosan-activated serum as chemotactic factor is not increased.

DISCUSSION

Microabscesses at the tips of dermal papillae characterize DH skin lesions by histopathology. The accumulation of PMN seems to be the result of PMN migration towards a chemotactic factor gradient localized in the tips of dermal papillae in DH skin (7).

Migration depends on the presence of a chemoattractant at the site of inflammation and on the chemotactic responsiveness of the inflammatory cells (6).

We investigated in vitro-chemotactic responsiveness of PMN from DH patients in comparison with chemotactic responsiveness of PMN from normal healthy individuals. Our results show that chemotactic activity of circulating PMN from DH patients does not differ from chemotactic activity of circulating PMN from normal healthy individuals.

It appeared feasible to us to study chemotactic

responsiveness of PMN in sulphone-treated patients, since previous investigations had indicated that therapeutic doses of sulphones do not influence the chemotactic responsiveness of PMN to chemoattractants *in vitro* (4, 12).

It has been hypothesized that PMN accumulation in DH skin is due to an increased quantity of chemotactic factors released at the site of the inflammatory process. *In vivo* bound IgA deposits in the tips of dermal papillae in DH skin activate complement (C) through the alternative pathway. In support of this theory, the third component of complement (C3), components of the alternative pathway (properdin and factor B) as well as C5 are frequently present in the same location as IgA (7). When complement is activated, complement-derived proteins such as C3a, C5a and C567 complex are formed. These proteins represent potent endogenous chemotactic factors (6) and are therefore able to draw the inflammatory cells into the area. It is likely that C5a which represents the most potent chemoattractive complement fraction (6) is responsible for the influx of PMN into DH skin.

In most cases DH is associated with gluten-sensitive enteropathy. The enteropathy as well as skin lesions improve when gluten-free diet is instituted (7). Consequently it has been postulated that gluten proteins could be present in the skin and might be responsible for the induction of inflammation. These proteins are potent cytotoxins for human PMN (8) but their presence in DH skin could not be demonstrated in previous studies. (10)

With regard to our data, we assume that PMN accumulation in skin lesions in DH is not due to increased chemotactic responsiveness of the cells; but is probably due to an increased number of chemotactic factors in this area. The nature of the chemotactic factors in DH skin is still not established, but seems rather to involve complement-derived proteins and not gluten proteins. Further studies using skin extracts from DH patients, as already done in psoriasis (9), will be necessary to define the nature of chemotactic factors located in DH skin.

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