

STUDIES ON Fc-RECEPTOR-BEARING MONONUCLEAR LEUKOCYTES FROM PERIPHERAL BLOOD OF PATIENTS WITH DERMATITIS HERPETIFORMIS

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Abstract. Isopaque-Ficoll separated mononuclear cell suspensions from peripheral blood of patients with dermatitis herpetiformis (DH) and healthy controls were investigated by means of a rosette assay for Fc-receptor-bearing leukocytes (EA-RFC), peroxidase staining for monocytes (Pox) and a plaque formation assay (PFC) as well as a ^{51}Cr release assay for cell-mediated cytotoxicity. The cell suspensions were investigated both before and after fractionation on nylon fibre columns. In the patients the mean percentage of PFC in unfractionated cell suspensions was significantly higher than in the controls. In fractionated cell suspensions both the mean percentage of EA-RFC and the mean cytotoxicity index in the ^{51}Cr release assay were significantly lower than in the controls. There were no differences in the percentages of PFC- and Pox-positive cells in fractionated cell suspensions. The results suggest a numerical defect of circulating Fc-receptor-bearing lymphocytes as estimated both by the rosette assay and the ^{51}Cr release assay. These data may reflect a pathogenetic role of these lymphocytes in DH.

Key words: Dermatitis herpetiformis; Immunopathogenesis; Fc-receptor-bearing cells

Dermatitis herpetiformis (DH) is today accepted as a disease with skin lesions associated with gluten-sensitive enteropathy in the vast majority of the patients (8). Recent years' research has focused on immunological abnormalities in this disease, and studies on immunoglobulins (4, 10, 17), the complement system (9, 11, 12) and the role of immunogenetic factors (7, 14) have been performed.

One of the major histopathological features of the inflammatory infiltrate in the gut is a predominance of lymphoid cells (13), while the skin lesions present intrapapillary micro-abscesses with neutrophils, subepidermal blisters, and lymphohistiocytic infiltrates surrounding dermal blood vessels (8). In a recent study on lymphocyte populations in DH, Fc-receptor-bearing lymphocytes were also studied

(1). The purpose of this investigation was to further investigate such leukocytes from peripheral blood of patients with dermatitis herpetiformis.

MATERIALS AND METHODS

Seventeen patients, age 26-70 years, with dermatitis herpetiformis (DH), and 13 healthy adults aged 26-69 years, were investigated. All the patients presented skin lesions at the time when blood was obtained, and none adhered to gluten-free diet or took sulphones the last 2 days prior to the investigation.

Sources of mononuclear leukocytes

Venous blood containing 10 units of heparin per ml was mixed with equal volumes of saline and centrifuged on an Isopaque-Ficoll gradient as described earlier (2). After being washed three times in Hanks' Balanced Salt Solution (HBSS) (Grand Island Biological Co., Grand Island, New York), the cells obtained from the interphase were suspended in RPMI 1640 (GIBCO, Grand Island) with 2.5×10^6 mononuclear leukocytes per ml for the plaque assay and in Medium 199 (GIBCO) with 5×10^6 mononuclear leukocytes per ml for the ^{51}Cr release assay. The culture media were prepared with penicillin (50 units/ml) and streptomycin (50 $\mu\text{g/ml}$).

Detection of mononuclear leukocytes with receptors for IgG (EA-RFC)

Rosette formation with ORh+ red cells sensitized with anti-Rh IgG antiserum (Ripley) was used as an assay system for these cells (5).

Histochemical studies

Preparations were made with a cytocentrifuge (Shandon Scientific Co. Ltd., London) and stained with peroxidase for determination of the peroxidase activity of the mononuclear leukocyte fractions (6).

Depletion of adherent cells

The depletion of adherent cells (B-lymphocytes, monocytes, granulocytes) was performed essentially as described previously (18). Approximately 1×10^6 cells in 15 ml Medium 199 with fetal calf serum were put on a 30 ml

Table I. The percentages of plaque forming mononuclear leukocytes (PFC) and peroxidase positive cells (Pox) from patients with dermatitis herpetiformis compared with the corresponding values from normal donors.

The results are presented as mean $\pm$ standard error of the mean

	PFC				Pox			
	n ^a	Ly ^b	n	Ly-C ^c	n	Ly	n	Ly-C
Patients	16	7.8 ($\pm$ 1.12)	6	<0.2	14	19.1 ($\pm$ 2.71)	6	1.0 ($\pm$ 0.35)
Controls	13	5.2 ($\pm$ 1.35)	13	<0.2	10	13.3 ($\pm$ 3.89)	10	<0.5
Difference patients versus controls	$p=0.0089$		n.s. ^d		n.s.		n.s.	

^a n: number of experiments performed.

^b Ly: Mononuclear leukocytes prepared by Isopaque-Ficoll gradient centrifugation.

^c Ly-C: Mononuclear leukocytes prepared by Isopaque-Ficoll gradient centrifugation and filtered through a nylon fibre column.

^d n.s.: not significant.

glass column containing 3.5 g nylon fibres (Fenwal Lab., Morton Grove, Ill.). After incubation of the column for 30 min at 37°C, elution was performed with 30 ml of Medium 199. The recovery of the cells applied on the column was usually about 50%.

Cytotoxicity assays

Plaque assay. The plaque assay was based on a procedure which is described in detail elsewhere (15), with a slight modification. A monolayer of sheep erythrocytes (SRBC) was made in poly-L-lysine-treated tissue culture cluster plates 24 (Costar 3524; Costar, Cambridge, U.K.). For induction of plaque formation, 0.5 ml of 1×10^{-4} diluted rabbit anti-SRBC antiserum or 0.5 ml RPMI 1640 (control cultures) was dripped slowly onto the monolayer before 1.25×10^5 mononuclear leukocytes were added to each plate and incubated for 20 hours in an atmosphere of 5% CO₂ and 100% humidity. By means of a grid fitted in the ocular of a phase contrast microscope the number of plaques measured in a randomly chosen area (2 mm²) was counted. An area corresponding to 10–15 lysed SRBC was defined as a plaque.

Chromium release assay. The method applied has been described elsewhere (18). Briefly, 1×10^5 ⁵¹Cr-labelled chicken erythrocytes (CRBC) in 0.5 ml of Medium 199 were mixed with an equal volume of 1.25×10^6 mononuclear leukocytes in round-bottomed tissue culture tubes, 15 $\times$ 100 mm (Bellco Glass Inc., Vineland, New Jersey). 0.5 ml of heat-inactivated rabbit anti-CRBC antiserum diluted 10^{-4} and 0.05 ml of fetal calf serum were added. Incubation was carried out for 20 hours at 37°C in an atmosphere of 5% CO₂ and 100% humidity. After incubation and centrifugation at 400 g for 10 min the radioactivity of the supernatant and the total radioactivity of each tube were determined in a gamma ray counter (Nuclear Enterprises, U.K.). The cytotoxicity index (CI) was calculated:

$$CI = \frac{\text{radioactivity of the supernatant}}{\text{total radioactivity}} \times 100.$$

Statistical method. Mann-Whitney's test.

RESULTS

Unfractionated cells.

In Isopaque-Ficoll separated mononuclear cell suspensions from patients with dermatitis herpetiformis there was a statistically significant increase in the mean percentage of plaque-forming cells (PFC) as compared with the controls (Table I). The mean percentage of EA-RFC was 17.4 in the patient group and 14.1 in the control group (Fig. 1). This difference is not significant, however. Furthermore, no significant differences were found between the two groups concerning the mean percentage of peroxidase-positive cells (Table I), and cell-mediated cytotoxicity as determined by the ⁵¹Cr release assay (Fig. 2).

Fractionated cells

After nylon column fractionation of cell suspensions, the percentage of EA-RFC in the patient group, 7.3%, was significantly lower than in the control group, 15.9% ($p=0.01$) (Fig. 1). Practically all PFC- and Pox-positive cells, both in the patient group and in the control group, were retained on the nylon fibre column (Table I). The cells from the patient group, however, showed a significantly decreased cytotoxicity in the ⁵¹Cr release assay on comparison with the controls ($p=0.0007$) (Fig. 2).

The decrease in mean EA-RFC in the patient group from 17.4% to 7.3% after the nylon fibre fractionation was significant ($p=0.01$), while no such difference was found in the control group (Fig. 1). Furthermore we observed a reduction in mean cytotoxicity index ($p=0.0009$) in the patients and an

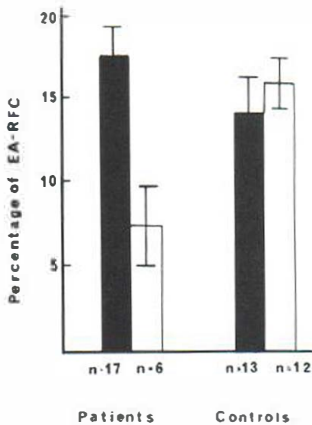


Fig. 1. Mean percentages of EA-RFC in cell suspensions before (black) and after (open) nylon fibre fractionation in patients with dermatitis herpetiformis and healthy controls. The results are presented as mean $\pm$ standard error of the mean. n = number of experiments performed.

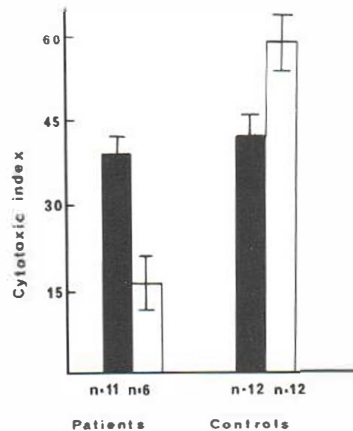


Fig. 2. Mean cytotoxicity indices in cell suspensions before (black) and after (open) nylon fibre fractionation in patients with dermatitis herpetiformis and healthy controls. The results are presented as mean $\pm$ standard error of the mean. n = number of experiments performed.

increase ($p=0.01$) in the controls after the nylon fractionations (Fig. 2).

DISCUSSION

The present investigation demonstrates various abnormalities in the mononuclear leukocyte cell populations in peripheral blood from patients with dermatitis herpetiformis. In unfractionated cell suspensions there was an increase in PFC in the patient group. We have previously presented data indicating that these cells have a monocytic origin, and are different from the cells responsible for antibody-dependent cytotoxicity in the ^{51}Cr release assay (15, 16). The function of PFC *in vivo* is not known and the possible importance of their increase in DH therefore remains unclear.

The increased numbers of monocytic cells involved in the plaque assay was paralleled by an increase in leukocytes positive with Pox staining, which is thought to be a criterion for monocytes, even though the increase was not statistically significant. After nylon column fractionation, the percentage of EA-RFC in the patients fell, from 17.4% to 7.3%. Normally the bulk of EA-RFC are non-adherent lymphocytic cells which are able to pass through a nylon column (5). In DH, however, a considerable proportion of the EA-RFC were adherent, thus displaying monocyte characteristics. The contribution of monocytic cells to EA-rosette formation in dermatitis herpetiformis was greater than in the controls, which was not unexpected in

view of our other findings indicating increased levels of monocytes in peripheral blood, i.e. increased levels of plaque-forming and peroxidase-positive cells.

In contrast to the increase in numbers of monocytic cells found in the various assays, the proportion of true Fc-receptor-bearing lymphocytes according to previously described characteristics (5) was reduced, as shown after depletion of the adherent cells. Similarly a reduction was seen in the lymphocyte cytotoxicity determined by the ^{51}Cr release assay. In contrast, the normal controls showed, as previously published (18, 19), an increased lymphocyte cytotoxicity after removal of adherent cells. The effector lymphocytes in this cytotoxicity assay are thought to represent a subpopulation within the heterogeneous population determined with the EA-RFC assay. Thus, both the EA-RFC assay and the functional cytotoxicity assay indicate a defect in the non-T-, non-B-lymphocyte population. This defect may be numerical, but our data does not permit us to exclude the possibility that qualitative changes in the cell membrane of these lymphocytes are the reason for the abnormalities.

The described abnormalities in the cytotoxic cells may be of relevance for the pathogenesis in DH. The Fc-receptor-bearing effector cells may contribute to the tissue damage in both the intestinal tract and the skin lesions. In this connection it is interesting that Ezeoki et al. (3) have shown that serum of

patients with gluten-sensitive enteropathia can arm lymphocytes to become cytotoxic effector cells for α -gliadin-coated erythrocytes used as targets.

The hypothesis that the Fc-receptor-bearing cytotoxic cells are involved in the pathogenesis of the lesions in DH might explain the decreased number of Fc-receptor-bearing lymphocytes in peripheral blood, which would then reflect an accumulation of these cells in the diseased gut and skin.

Monocytes, however, may also contribute to the tissue damage in DH, and the increased number of circulating monocytic cells demonstrated may therefore be relevant for the pathogenesis.

Further studies are necessary to elucidate the pathogenic role of Fc-receptor-bearing cells in dermatitis herpetiformis.

ACKNOWLEDGEMENTS

We are grateful for the excellent technical assistance of Mrs Bodil Lunden. L. Thorsteinsson is a research fellow of Norwegian Research Council for Science and the Humanities.

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Received October 28, 1978

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