

Increased IgG Fc and C3 Rosette-Forming Capacity of Granulocytes from Patients with Psoriasis

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In the present work the proportions of IgG Fc and C3 rosette-forming granulocytes were studied in patients with severe psoriasis. The percentages of erythrocyte-antibody and C3 rosette-forming granulocytes from psoriasis patients were significantly higher than those of healthy individuals. Sera from patients with psoriasis did not influence the rosette formation by granulocytes from healthy persons. The increased IgG Fc and C3 receptor activities of granulocytes in psoriasis may account for the hyperactivity of these cells enhancing the susceptibility of the polymorphonuclears for at least some naturally occurring stimuli.

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The presence of polymorphonuclear leukocytes (PMN) in the epidermal infiltrate at the onset of psoriatic lesions (1), and the reported alterations in the functional activities of circulating PMN (3, 4, 5, 7, 8, 11), suggest that PMN may participate in the pathogenesis of psoriasis. The mechanisms whereby the abnormalities of phagocyte function arise are far from clear. The PMN functions are dependent on the interactions occurring on the cell surface membrane. Membrane receptors regulate both signal perception, signal processing and the effector functions of the phagocytes.

In the present work, two granulocyte membrane receptors, the IgG Fc and C3, were investigated with rosette techniques in patients with psoriasis. The effects of sera from severe psoriasis patients on the IgG Fc and C3 receptors of granulocytes from healthy individuals were also studied.

PATIENTS AND METHODS

The 19 patients involved in the study (10 males and 9 females, aged 22-67 years) suffered

from plaque-type psoriasis, affecting more than 25% of the skin surface. The patients had received no treatment during the previous 3 months. Controls were 18 healthy volunteers (12 males and 6 females, aged 18-73 years).

PMN were separated from heparinized fresh venous blood by dextran sedimentation (4). The viability of the cells was determined with the trypan blue exclusion test. More than 95% of the cells were living granulocytes.

Erythrocyte-antibody (EA) rosetting test

Bovine erythrocytes ($100 \mu\text{l}$ of 2×10^6 cells/ml phosphate-buffered saline PBS, pH 7.2) were sensitized with $100 \mu\text{l}$ rabbit anti-bovine red blood cell IgG in subagglutinating concentration. $100 \mu\text{l}$ of sensitized erythrocytes (2×10^6 /ml PBS) was added to $100 \mu\text{l}$ granulocyte suspension (5×10^6 cells/ml Parker TC 199 medium). The cell mixture was incubated for 5 min at 37°C and then centrifuged for 1 min at $400 g$. The tubes were stored at 4°C for 1 h. The granulocytes which bound at least three erythrocytes were counted as rosettes in a conventional light microscope.

C3 rosetting test

The C3 rosetting test was carried out with complement-coated zymosan beads using a modified method described by Huber & Wigzell (6). 300 μ l of zymosan beads (Sigma, St. Louis, USA), (6×10^7 beads/ml PBS) was incubated with 100 μ l pooled fresh human serum at 37°C for 30 min. After double washing of the beads, 100 μ l granulocyte suspension (10^6 cells/ml Parker TC 199 medium) was added to 200 μ l zymosan suspension (5×10^7 particles/ml). The tubes were centrifuged for 4 min at 400 g and incubated for 5 min at 37°C. After gently shaking the tubes the rosettes were immediately counted as described above. The granulocytes which bound at least three zymosan beads were considered as rosettes.

Effects of psoriasis serum on rosette formation by granulocytes from healthy persons

Sera from 10 untreated patients with widespread psoriasis were collected in equal proportions and stored for no longer than 1 week at -20°C. 200 μ l granulocyte suspension (10^7 cells/ml Parker medium) from each of the 18 healthy volunteers was incubated with 200 μ l mixed psoriasis serum for 30 min at 37°C. After double washing of the granulocytes, the rosetting tests were carried out as described above.

Statistical calculations were performed with the Welch-test (EA rosetting test) and the Student's t-test (C3 rosetting test).

RESULTS

The percentage of EA rosette-forming granulocytes from psoriasis patients was 63.8 ± 9.85 , compared with 50.25 ± 3.73 in the control group ($P < 0.01$). The percentage of the C3 rosette-forming granulocytes was similarly significantly higher in the psoriasis group (75.2 ± 5.31) than in the healthy group (62.9 ± 9.94) ($P < 0.005$). The data are listed in Table 1.

Control studies were carried out, in which rosette formation by PMN was tested with bovine erythrocytes without sensitization and with complement-coated zymosan particles subsequently inactivated by heat (56°C, 1 h): the percentages of rosette-forming granulocytes were similar in the psoriasis and the healthy groups. With non-sensitized bovine erythrocytes the percentages were lower than 5%, while the percentages of granulocytes capable of rosette formation with complement-coated, heat-inactivated zymosan beads were lower than 8% in each case.

Neither rosette formation, using IgG-sensitized bovine erythrocytes or complement-coated zymosan beads, was influenced when the granulocytes from healthy individuals were preincubated with psoriasis sera (Table 2). The

Table 1

Percentages of IgG-sensitized bovine erythrocyte (EA) and C3 rosette-forming granulocytes in patients with psoriasis and in healthy individuals

	n	Percentage of EA rosette-forming granulocytes	n	Percentage of C3 rosette-forming granulocytes
Psoriasis patients	9	63.8 ± 9.85	10	75.2 ± 5.31
Healthy persons	8	50.25 ± 3.73	10	62.9 ± 9.94
<i>P</i> <		0.01		0.005

Granulocytes which bound at least three IgG-sensitized erythrocytes or complement-coated zymosan beads were counted as rosettes, using a conventional light microscope. The tabulated data are means $\pm$ SD. Statistical calculations were carried out with the Welch-test (EA rosetting test) and the Student's t-test (C3 rosetting test).

Table 2

Effects of psoriasis sera on the IgG-sensitized bovine erythrocyte (EA) and C3 rosette formation of granulocytes from healthy individuals

	n	Percentage of EA rosette-forming granulocytes	n	Percentage of C3 rosette-forming granulocytes
Granulocytes without preincubation	8	50.25 $\pm$ 3.73	10	62.9 $\pm$ 9.94
Granulocytes preincubated in psoriasis sera	8	50.86 $\pm$ 9.85	10	59.0 $\pm$ 5.12
P		N.S.		N.S.

Granulocytes were preincubated for 30 min at 37°C. with sera collected in equal proportions from 10 severe psoriasis patients. Rosette techniques were carried out as described in the text. Percentages are those of granulocytes which bound at least three sensitized erythrocytes or complement-coated zymosan beads. The tabulated data are means $\pm$ SD. Statistical calculations were performed with the Welch-test (EA rosetting test) and Student's t-test (C3 rosetting test).

percentages of EA rosette-forming and C3 rosette-forming granulocytes remained practically unchanged after preincubation with psoriasis serum: 50.86 $\pm$ 9.85 and 59.0 $\pm$ 5.12%, respectively. The corresponding data from Table 1 are not statistically significantly different.

DISCUSSION

In the present work we report that higher proportions of granulocytes from patients with severe psoriasis bind IgG-sensitized bovine erythrocytes and complement-coated zymosan beads than do those of healthy individuals. The differences observed between the psoriasis and the healthy groups are receptor-dependent: in control experiments where rosette formation of granulocytes with bovine erythrocytes without sensitization and with complement-coated, heat-inactivated zymosan particles was studied, the percentages of rosette-forming PMN were similar in the two groups. The data reveal increased IgG-Fc and C3 receptor activity (expressivity and/or avidity) of the granulocytes in psoriasis. Preincubation of granulocytes from healthy persons with sera from psoriasis patients failed to influence the activity of these receptors. This observation indicates that serum

factors play no, or only a minor, role in the development of the altered receptor activity.

In the past few years many investigators have demonstrated an enhanced PMN function in psoriasis. However, little is known about the mechanisms involved in the development of the functional alterations. Recent studies seemingly rule out the dysregulation of the PMN function by cyclic nucleotides in psoriasis (5). The granulocyte function abnormalities are strongly dependent on the cell surface membrane receptors, which perceive and mediate the signals regulating the phagocytes. Chemotaxis (4, 7, 8, 11), antibody-dependent cellular cytotoxicity (5), phagocytosis (11), all of which have been extensively studied in psoriasis, are initiated by signal recognition with surface receptors. Complement levels are significantly elevated in psoriasis patients (9), and circulating immune complexes have also been identified (2). Sedgwick et al. (10) considered that the increased percentage of granulocytes with abnormal morphology in psoriatics was due to the influence of chemotactic factors. The increased Fc and complement receptor activity of the PMN in psoriasis may account for an increased cell susceptibility to at least some naturally occurring stimuli, and may thus explain the morphological and functional abnormalities of the psoriatic phagocytes.

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