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Fc receptors on granulocytes from patients with rheumatoid arthritis and Felty's syndrome

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SUMMARY

Receptors for the Fc part of IgG on polymorphonuclear cells (FcR) of patients with rheumatoid arthritis (RA), Felty's syndrome (FS) and healthy controls (HC) were studied by means of a rosetting technique with rabbit IgG coated ox erythrocytes. When cold isolated polymorphonuclear cells (PMN) were incubated at room temperature the percentage of rosette forming PMN (RF-PMN) from HC was more than twice that measured directly after isolation at 4°C. The same phenomenon was observed for PMN from patients with RA although the RF-PMN increased by only 1/3. In contrast warming of PMN from patients with FS did not influence the RF-PMN. The presence of surface bound immunoglobulins and intracytoplasmic immunoglobulins was measured by immunofluorescence and appeared to be inversely related to the RF-PMN. A good correlation between the results of the C1q binding assay (C1qBA) and the immunofluorescence score was observed but no correlation existed between the C1qBA and the RF-PMN. These results indicate that the number of PMN expressing FcR in patients with RA and FS is decreased presumably because of the phagocytosis of immune complex like material. The decrease in the availability of FcR may influence the functions of the PMN.

Keywords Fc receptors rheumatoid arthritis Felty's syndrome

INTRODUCTION

Soluble immune complexes (IC) and IgG coated particles can be bound to the membrane of several white blood cells by means of receptors (FcR) for the Fc part of IgG (Henson, 1968; Messner & Jelinek, 1970). Cellular responses following the occupation of the receptor binding site include phagocytosis, digestion and a decreased ability of neutrophils to migrate. The capacity of cells, such as monocytes and polymorphonuclear cells (PMN) to perform phagocytosis and digestion is dependent on the number of cells expressing functionally active FcR (Scribner & Fahrney, 1976; Daha & Van Es, 1982; Leyh *et al.*, 1980). In a recent study Fearon & Collins (1983) demonstrated that the availability of receptors for the third component of complement (C3bR) increased markedly when PMN isolated on ice were brought to room temperature. These authors suggested that this phenomenon could be explained either by a change in conformation, thus making new binding sites available, or, more likely, enhanced expression of receptors on the membrane.

Impaired functioning of PMN, e.g. abnormal chemotaxis, phagocytosis or digestion, has been reported in patients with rheumatoid arthritis (RA) and Felty's syndrome (FS) (Howe *et al.*, 1981; Hällgren, Hakansson & Venge, 1978; Gupta, Laforce & Miles, 1975; Bültman *et al.*, 1980). It has

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been suggested that this phenomenon could be due to phagocytosis of IC by these cells, as already described in these diseases (Cats, Lafeber & Klein, 1975; Hurd, 1978). Since the uptake of IC is mediated by membrane receptors it could be possible that the expression of such receptors is impaired in these diseases. In the present study, we investigated the differences in the number of PMN expressing FcR between healthy controls (HC), patients with RA and FS. The expression of FcR is measured by the percentage of PMN forming rosettes (RF-PMN). The amount of surface bound immunoglobulins (SIg), intracytoplasmic immunoglobulins (CIg) and circulating IC in the sera of these patients were compared to FcR expression.

MATERIALS AND METHODS

Patients. Peripheral blood was obtained by venapuncture from 15 patients with definite RA according to the ARA criteria, six patients with FS (RA with or without splenomegaly, neutrophil count of less than $2{\cdot}0 \times 10^9$/l) and six healthy controls (HC). All patients had positive Waaler–Rose and latex fixation tests. None of the patients received corticosteroids or cytostatic drugs at the time of the investigation.

PMN isolation. Twenty millilitres of heparinized venous blood and 1·6 ml 0·129 M sodium citrate were placed on ice for at least 15 min. All subsequent steps were performed on ice using cold materials. After four times diluting with PBS, blood was brought on 5 ml dextran cushions and the erythrocytes allowed to settle at unit gravity for 30 min. The leucocyte rich supernatant was layered on Ficoll-Hypaque (density = 1·077 g/cm^3) and centrifuged for 20 min at 900*g* on ice. The residual erythrocytes in the pellet were lysed with 0·85% bicarbonate-buffered NH_4Cl (pH 7·2). The remaining PMN were washed three times with PBS containing 1·6 mg glucose per ml and resuspended to a concentration of 10×10^6 cells/ml. The preparations contained over 90% granulocytes, including 2–15% eosinophils. Viability, tested by trypan blue exclusion, appeared to be more than 95%.

Quantity of cells bearing Fc receptors. The presence of Fc receptors on PMN was demonstrated by means of a rosetting technique using ox erythrocytes coated with rabbit IgG anti-ox erythrocytes (Eox A) (Cnossen *et al.*, 1980). Cold isolated PMN were assessed for the number of rosette forming cells, directly, after 1 h standing at room temperature or after various periods of incubation at 37°C. Counting of the rosette forming cells was performed in the cold as follows: to 100 μl of PMN (10×10^6/ml) in 675 μl PBS containing 75 μl fetal calf serum, 225 μl of an Eox A suspension (2×10^8 Eox A/ml) were added. After centrifugation at 200*g* at 4°C one drop of brilliant cresyl blue was added and the cells were carefully resuspended. The rosettes were counted directly in a haemocytometer. PMN were considered as a rosette forming cell when two or more erythrocytes were attached. The RF-PMN of one HC measured on 4 subsequent days and varied < 7%.

Demonstration of surface Ig (SIg) on viable PMN. Immunofluorescence studies were performed on ice. One million PMN were incubated with 50 μl of diluted FITC labelled $F(ab')_2$ fragments of goat anti-human IgG (1 : 40), IgM (1 : 50), IgA (1 : 10) or C3 (1 : 15) (Kallenstad, Austin, Texas, USA) on ice for 30 min. After washing three times with ice cold PBS the PMN were immersed in a drop of 90% buffered glycerol and examined directly under a fluorescence microscope. Fluorescence was expressed semiquantitatively in four grades: 0 = no fluorescence; 1 = borderline or faint fluorescence; 2 = clear fluorescence in lumps or rings; 3 = strong rings or lumps of fluorescence. At least 300 cells were scored. The percentage of cells within each grade was multiplied by that grade number and the amount of fluorescence score for each experimental condition was expressed as the sum of these products. Fluorescence on PMN from HC was positive for IgG (score < 100) and negative for IgM and IgA.

Demonstration of intracytoplasmic immunoglobulins (CIg) on fixed PMN. Cold isolated PMN were cytocentrifuged. After air drying at room temperature the slides were fixed in 96% alcohol containing 5% acetic acid at 20°C for 15 min and then washed in PBS at 4°C for 1 h. FITC labelled goat anti-human IgG (Fc, dilution 1 : 30) was purchased from Nordic Immunological Laboratories (Tilburg, The Netherlands). FITC labelled heavy chain specific rabbit antibodies against IgA (1 : 45), IgM (1 : 30) and C3c (1 : 15) were obtained from Dakopatts (Copenhagen, Denmark). These

antisera were added to the preparations and incubated in a moist chamber at room temperature for 30 min. After washing for 10 min in PBS the preparations were immersed in a drop of 90% glycerol and fluorescence was scored semi-quantitatively as follows: 0 = no fluorescence; 1 = small inclusions; 2 = more, clear inclusions; 3 = many, large inclusions. Immunofluorescence score was expressed as described above for SIg. The score of PMN from HC was < 10.

IC. For the detection of circulating IC in serum the C1q binding assay (C1qBA) was used (Zubler *et al.*, 1976). The amount of IC was expressed as μg equivalents of a standard of aggregated human IgG (Kauffman, Van Es & Daha, 1979).

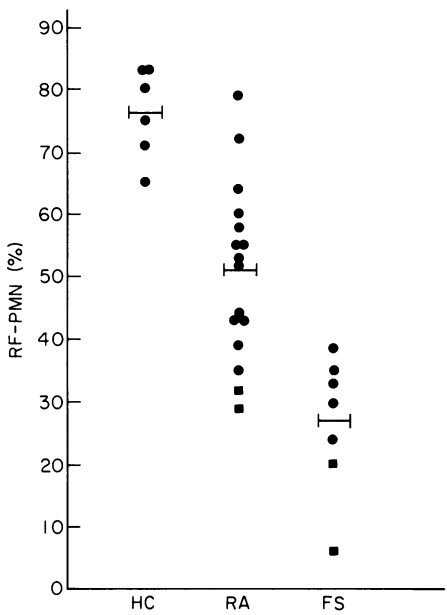

Fig. 1. Percentage of RF-PMN from HC and patients with RA and FS. ■ = patients measured at two occasions. The mean of each group is indicated by a bar.

RESULTS

Rosette forming cells after incubation at room temperature

The RF-PMN obtained from HC and patients with RA and FS were determined using suspensions of cold isolated cells incubated for 1 h at room temperature (Fig. 1). The mean of the percentages for the HC group was 76·2% (s.d. = 7·2), which differs significantly ($P < 0{\cdot}001$) from the percentage for the group of RA patients (50·8%, s.d. = 14·1). The percentage for patient with FS (26·4%, s.d. = 11·0) was significantly lower than that for the RA group ($P < 0{\cdot}001$). In both patient groups one patient was measured on two occasions.

Temperature dependence of the percentages of RF-PMN

To investigate the effect of temperature on the rosette formation of cold isolated PMN the cells were incubated for either 0, 15, 30, 45 or 60 min at 37°C and subsequently assessed for rosette formation at 4°C (Fig. 2). For HC the percentage of RF-PMN was twice as high after 15 min at 37°C measured directly after isolation at 4°C (66% *vs* 28%). PMN from patients with RA also showed a significant increase ($P < 0{\cdot}001$) in the percentage of rosette forming cells after 15 min at 37°C, although the increase was smaller (47% *vs* 34%). In contrast, incubation of PMN from patients with FS for 15 min at 37°C did not result in an increase of RF-PMN. Prolonged incubation at 37°C caused no further increase in the RF-PMN compared to the initial 15 min.

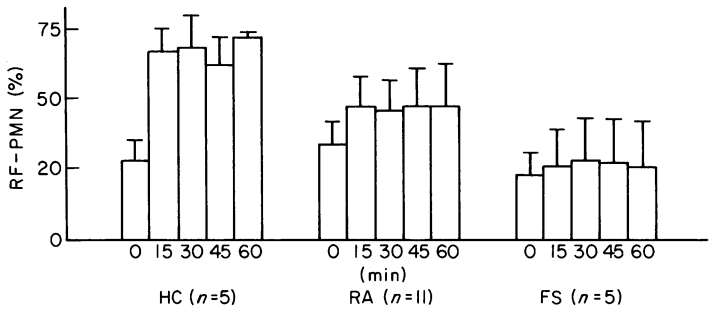

Fig. 2. Percentage of RF-PMN from HC, patients with RA and FS after incubation for several periods at 37°C. Each column represents the mean of each group and the standard deviations are depicted as well.

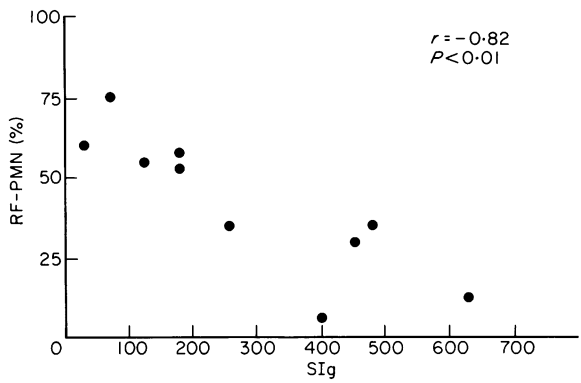

Fig. 3. Correlation between the percentage of RF-PMN and the sum of the scores for surface IgG, IgA and IgM on the PMN (SIg).

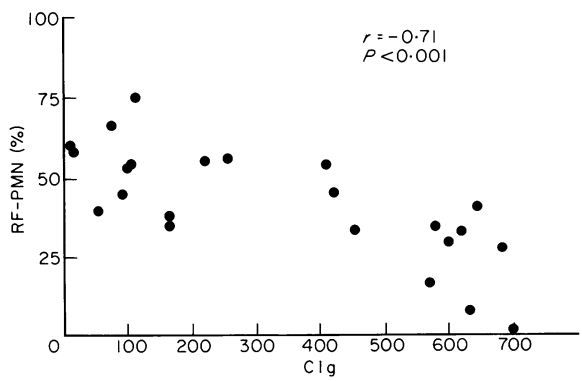

Fig. 4. Correlation between the percentage of RF-PMN and the sum of the scores for intracytoplasmic IgG, IgA and IgM (CIg).

Comparison of rosette forming cells and the presence of SIg and CIg.

The presence of SIg (determined for IgG, IgM and IgA) on PMN appeared to be inversely related to the percentage of rosette forming cells measured after incubation at 37°C for 15 min. Since all three classes of Ig revealed the same profile in relation to rosette formation the sum of the score for each class was related to the percentage of PMN bearing rosettes (Fig. 3). An inverse relationship was

Table 1. Linear regression analysis of the percentages of RF-PMN, the immunofluorescence score on PMN SIg or CIg and the results of the C1qBA for patients RA or FS

	RA+FS			RA			FS		
	r	*P*		*r*	*P*		*r*	*P*	
C1qBA *vs* SIg	0·63	<0·01	(*n*=10)	0·71	<0·05	(*n*=6)	0·74	NS	(*n*=4)
C1qBA *vs* CIg	0·62	<0·001	(*n*=23)	0·56	<0·05	(*n*=16)	0·85	<0·01	(*n*=7)
C1qBA *vs* RF-PMN	0·30	NS	(*n*=23)	0·41	NS	(*n*=16)	0·23	NS	(*n*=7)
SIg *vs* RF-PMN	−0·82	<0·01	(*n*=10)	−0·94	<0·001	(*n*=6)	−0·09	NS	(*n*=47)
CIg *vs* RF-PMN	−0·71	<0·001	(*n*=23)	−0·72	<0·001	(*n*=16)	−0·60	NS	(*n*=7)

NS = not significant; *r* = correlation coefficient.

found between these two parameters ($r = -0{\cdot}82$; $P < 0{\cdot}01$). An identical picture (Fig. 4) was obtained for CIg under these experimental conditions ($r = -0{\cdot}71$; $P < 0{\cdot}001$). The correlations for patients with RA and FS are given in Table 1. No correlation existed between either surface bound or intracytoplasmic C3 and RF-PMN, SIg or CIg.

Comparison of rosette forming cells, SIg, CIg and circulating IC

The levels of circulating IC measured by the C1qBA were compared with the fluorescence scores for SIg and CIg positive cells and with the percentages of rosette forming PMN (Table 1). Linear correlations were found between the results of the C1qBA and the scores for surface bound IgG ($r = 0{\cdot}69$; $P < 0{\cdot}001$) and cytoplasmic IgG ($r = 0{\cdot}62$; $P < 0{\cdot}05$). Moreover the scores for total SIg and CIg correlated with the results of the C1qBA ($r = 0{\cdot}63$; $P < 0{\cdot}01$ and $r = 0{\cdot}62$; $P < 0{\cdot}001$). In contrast the results of the C1qBA compared with the RF-PMN revealed that no correlation existed.

DISCUSSION

The results of the present study demonstrate that PMN from patients with RA and FS have less FcR available on the plasma membrane. Fearon & Collins (1983) showed that the low number of C3b receptors (C3bR) expressed on PMN after isolation on ice represents the situation *in vivo* with increased numbers of receptors becoming apparent at temperatures between 20 and 37°C. Under similar conditions our experiments show the same phenomena for FcR. This means that both HC and patients with RA and FS have a low expression of FcR on granulocytes *in vivo* but the patients have a decreased ability to increase this expression at higher temperature. The low percentage of rosettes found after incubation of cold isolated PMN from patients with RA and FS at 37°C was inversely related to the score for surface bound and cytoplasmic immunoglobulins. Moreover the scores for SIg and CIg were correlated with the amount of circulating IC. These findings are in agreement with the data obtained by Griffin (1980). This author suggested that internalization of the receptors after FcR-mediated phagocytosis impaired subsequent binding via these receptors. Starkebaum, Jiminez & Arend (1982) demonstrated that the addition of IC to PMN significantly diminished FcR function and that this effect was probably due to the accumulation of myeloperoxidase-mediated toxic products. In the light of these data, it can be suggested that the low percentages of rosettes formed by PMN from patients with RA or FS is likewise due to previous phagocytosis of IC *in vivo*. However the numbers of available FcR on PMN in the present study were not directly correlated with levels of circulating IC. This lack of correlation may be explained by several different mechanisms. Firstly, these IC detected by the C1qBA do not interact efficiently with FcR. Secondly, metabolic alterations after the phagocytosis of IC could lead to a loss of FcR. Thirdly, the IC within PMN could have been bound and phagocytozed from the affected tissues rather than the circulation. Fourthly, the blockade of FcR by leucocyte specific antibodies, as

described in RA and FS, can not be excluded (Rosenthal *et al.*, 1974; Starkebaum *et al.*, 1980). The detection of C3 in these phagocytozed complexes did not correlate with any of the immunoglobulins measured and therefore must be explained as a non-specific phenomenon. Similar non-specific binding of C3 in IC has been observed by Kauffman *et al.* (1983).

It has been reported that patients with FS and to a lesser extent RA are more likely to suffer from bacterial infections (Baum, 1971; Moore *et al.*, 1971). Studies have indicated that reduced chemotaxis of PMN, as reported for patients with RA and FS, is caused by a decreased availability of FcR, already occupied by IC (Kay, Bumol & Douglas, 1978; Howe *et al.*, 1981). Phagocytosis by PMN is dependent on opsonization of bacteria by IgG and C3b. Diminishing the number of available FcR may therefore result in a decreased FcR-mediated phagocytosis. This is suggested by the observations of Starkebaum *et al.* (1982) who showed that incubation of PMN with IC led to reduction in the subsequent ingestion of complexes. Hällgren *et al.* (1978) also showed a diminished uptake of IgG coated particles in patients with RA. However other investigators using Fc and C3b coated bacteria report a normal phagocytic rate in patients with RA and FS (Gupta *et al.*, 1975; Bültman *et al.*, 1980). Hed & Stendahl (1982) reported that occupation of the FcR in contrast to the C3bR induces a metabolic burst in the granulocytes. If the blockade of the FcR results in a less efficient metabolic burst after phagocytosis of bacteria this could explain the reported reduction in intracellular killing capacity in patients with RA and FS (Gupta *et al.*, 1975; Bültman *et al.*, 1980). Further experiments are in progress to investigate phagocytosis and intracellular killing of bacteria by PMN from patients with RA and FS.

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