

DEFECTIVE FUNCTION WITH NORMAL EXPRESSION OF CELL-SURFACE Fc RECEPTORS IN MIXED CRYOGLOBULINAEMIA

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Summary

To gain insight into the role of Fc receptors in essential mixed cryoglobulinaemia (EMC), we quantified their expression on polymorphonuclear cells (PMN). In addition the PMN IgG mediated phagocytosis, the macrophage immune clearance and the kinetics of phagocytosis of IgG-coated materials by monocytes were evaluated.

Despite a normal expression of PMN Fc receptors, six patients with EMC had depressed PMN phagocytosis, prolonged macrophage immune clearance with reduced monocyte kinetic index of phagocytosis.

Fc receptors are normally expressed but functionally impaired in EMC patients. These dysfunctions are likely to contribute to the tissue localization of immune material.

Introduction

Functional studies on phagocytic cells have recently stressed the role of these cells in removing immunologically active substances in systemic lupus erythematosus and other diseases characterized by tissue deposition of immune complexes [1]. These studies were based on the functional properties of the receptors for the Fc portion of IgG (Fc γ) of phagocytic cells, thought to mediate binding and processing of immune materials in the bloodstream.

When IgG autoantibodies are involved in immune complex formation, as in essential mixed cryoglobulinaemia (EMC), Fc γ receptor-mediated clearance might be important in the development of the disease process.

To gain insight into the role of Fc γ receptors in EMC-associated glomerulonephritis, we quantified the expression of these receptors on phagocytic cell surfaces by employing a mouse monoclonal autoantibody (MoAb) to Fc γ receptors of human polymorphonuclear cells (PMN), using rabbit F(ab)₂ fragments to mouse IgG labelled with ¹²⁵I for detection. Moreover, the functional activities of circulating and tissue phagocytic cells were examined in

in vitro or in vivo to assess their contribution to the clearance of immune materials in EMC.

Materials and methods

Patients Six Rh-positive patients (5 females and 1 male), mean age 53.2 years (range 38–68), typed for HLA-A, B, C, DR, DQ antigens and selected for the absence of DR3 antigen entered the study. Each patient was referred to the Nephrology Unit because of microscopic haematuria and/or proteinuria. In four cases the diagnosis of glomerulonephritis was confirmed by renal biopsy. Immunoelectrophoresis of the washed cryoprecipitate proved the presence of monoclonal IgM with Kappa light chain and polyclonal IgG. At the time of study four patients had severe microscopic haematuria (>25 red blood cells (RBC)/high power microscopic field) in three cases associated with nephrotic-range proteinuria. The other two patients had only minimal urinary abnormalities. With respect to the HLA-DR pattern, three patients were positive for the DR4 antigen, four for DR5, one for DR7.

Leucocyte isolation PMN from patients and controls were separated by Voss-Jepsen's one-step method [2] using two different gradients of Percoll (Pharmacia). Monocytes were isolated as described elsewhere [3] by employing density gradients of Percoll (Pharmacia P-L Biochemicals, Uppsala).

Evaluation of Fc receptor expression on the PMN surface A new MoAb, the AB8.28, obtained as described elsewhere [4] and rabbit ^{125}I F(ab)2 fragments to mouse IgG as a detecting system were employed. Specific activity was about $0.2\mu\text{Ci}/\mu\text{g}$. In the binding assays, aliquots of PMN suspension at 10^7 cells/ml were added to micro-test tubes. MoAb AB8.28 was added in slight excess, as determined in preliminary saturation experiments, and the mixtures were incubated for 60 minutes in a melting ice bath. After washing twice, about $0.18\mu\text{g}$ ^{125}I -labelled F(ab)2 anti-mouse IgG were added and incubation was continued for an additional 60 minutes. The cells were washed three times and the pellets collected in another series of test tubes and counted. Specific binding was determined by subtracting from the total binding counts bound to the cell pellet in the absence of MoAb.

PMN phagocytosis assay The Fc γ receptor activity of PMN was assessed by using ^{51}Cr -labelled sheep red blood cells coated with rabbit anti-sheep red blood cells. PMN were incubated with sheep red blood cells (ratio 1:50) for 60 minutes at 37°C in a 5% CO_2 incubator. After the incubation, PMN-bound sheep red blood cells were lysed with ammonium chloride-tris buffer and PMN washed three times with phosphate buffered saline (PBS). The radioactivity of the final pellet counted in triplicate in a gamma counter was related to the total activity of sheep red blood cell suspension previously incubated. This percentage value, divided for the number of PMN assayed, was considered as the 'phagocytosis index'.

In vivo clearance of IgG-sensitized autologous erythrocytes This assay was performed as previously described [5] by using Rh-positive autologous erythrocytes labelled with ^{51}Cr and sensitized with purified IgG anti-Rh (D). The half-life ($T_{1/2}$) of erythrocytes was calculated by a least square computing program with exponential fitting which relates the counts per minute in samples sequentially taken at 10 to 90 minutes after erythrocyte injection to the time.

Kinetic assay of phagocytosis of anti-Rh (D) IgG-coated red blood cells by circulating monocytes Identical aliquots of 0.3×10^6 OKM1 positive cells were tested for their ability to ingest, after diverse incubation times, autologous erythrocytes coated with IgG anti-Rh (D). The rate of phagocytosis was calculated – as previously described [6] – by determining the tangent to the angle $\alpha(\text{tg}\alpha)$ subtended by a regression line derived from plotting the number of erythrocytes ingested by 100 monocytes as a function of time.

Controls Twenty normal volunteers from the Medical and Laboratory personnel were examined as controls for the studies on macrophage immune clearance and 12 normal subjects served as controls for the kinetic assay of phagocytosis of monocytes. Eight normal females were studied for PMN phagocytosis and evaluated for Fc γ receptor expression.

Statistical methods Statistics were performed using Mann-Whitney two-tailed test.

Results

The specific binding of ^{125}I -labelled F(ab) $_2$ to AB8.28 MoAb bound to human PMN Fc γ receptors was 13.18ng/ 2.5×10^6 PMN (10.89–16.68) for patients and 13.44 (11.34–15.12) for controls. By assuming that one F(ab) $_2$ molecule binds one molecule of MoAb fixed to one Fc γ receptor, Schatchard transformation

TABLE I. Phagocytic cell Fc receptor-mediated function in EMC

	Patients	Controls
Polymorphonuclear leucocytes (phagocytosis index)	0.18 \pm 0.04* (0.13–0.21)	0.57 \pm 0.08 (0.52–0.67)
Splenic macrophages (clearance half-time of IgG-sensitized red blood cells)	61.6 \pm 33* (24–101)	32.9 \pm 10 (22–46)
Monocytes (kinetic index of phagocytosis IgG-coated red blood cells)	0.4 \pm 0.2** (0.1–0.75)	1.02 \pm 0.2 (0.8–1.3)

* $p < 0.05$; ** $p < 0.02$

[7] predicts a number of Fc γ receptors per cell in a patient of 31,644 \pm 5,020 (range 26,140–40,050), which was not significantly different from control values (mean 32,240 \pm 3,780, range 27,200–36,300). The features of PMN phagocytosis, macrophages' immune clearance and kinetics of phagocytosis of IgG-coated materials by peripheral monocytes are shown in Table I. As compared to normal subjects, patients had an impairment of functional activity of all phagocytic cells.

Discussion

We found a defective Fc receptor mediated clearance of immune material in patients with IgG-IgM mixed cryoglobulinaemia selected for the absence of HLA-B8, DR3 antigens, known to be associated with a primary impairment of this function [8]. This defect was found to involve all phagocytic cells despite the normal receptor cell surface expression as studied by detecting the PMN Fc γ receptors. The absolute number of Fc γ receptors calculated by our method must be regarded with caution due to the many assumptions involved in the employment of Scatchard analysis in an indirect technique using F(ab) $_2$ fragments directed to mouse IgG. PMN of patients with cryoglobulinaemia were frequently found to bind, likely by Fc γ receptors, IgG-linked immune material [9]. Therefore our findings of a normal cell Fc γ receptor expression associated with a defective Fc γ receptor-mediated phagocytosis are consistent with the hypothesis of an active involvement of circulating immunologically active substances in the development of the defective clearance of IgG-coated materials. These materials could directly saturate the Fc γ receptors or produce an exhaustion of cell energy source as supposed in monocytes from lupus patients [10], due to the continual immune complexes ingestion, finally accounting for the impaired phagocytic activity.

Whatever the mechanism involved, our data, even with a low number of patients, support the hypothesis that the general impairment of the phagocytic system in cryoglobulinaemia might be at least favoured by circulating immune materials, whose blocking effects on Fc γ receptor function might lead to a vicious circle of impaired clearance and increased glomerular disposition.

References

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