Wissenschaftliche Kurzmitteilung

Functional Fc-Receptor Defect of Polymorphonuclear Leukocytes in a Patient with Sjögren’s Syndrome*

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Summary. We investigated polymorphonuclear leukocyte (PMN) function in a 50-year-old woman with primary Sjögren’s syndrome (SS). The respiratory burst of PMN was monitored by luminol-enhanced chemiluminescence using zymosan, opsonized zymosan, zymosan-activated serum, and phorbol-myristate-acetate, as well as serial dilutions of aggregated immunoglobulin (aggIg) as Fc-receptor (FcR) stimulus. The effects of serum on the chemiluminescent response as well as the binding of aggIg to PMN were also compared. We found the patient’s PMN not to respond to stimulation by aggIg, only the highest concentration (> 180 μg/ml) induced a marginal chemiluminescent response in the patient. By contrast, incubation of the patient’s PMN with other stimuli resulted in responses similar to those in a healthy control. Binding of aggIg to PMN was higher in the patient (3.6% vs 1.5% of the radioactivity added in the control). Sera of patient and control induced similar chemiluminescence on PMN as did that of another human serum. Our data indicate a selective functional FcR defect of PMN despite unimpaired binding of aggIg in a patient with SS.

Key words: Fc-receptor – Polymorphonuclear leukocytes – Sjögren’s syndrome

A number of immunological abnormalities involving an increased association with the HLA haplotype B8/DRw3 have been described in Sjögren’s syndrome (SS) [1]. Individuals with this haplotype including patients with systemic lupus erythematosus (SLE), dermatitis herpetiformis Duhring (DHD), and healthy persons have been demonstrated by in vivo studies [2, 5] to exhibit defective Fc-receptor (FcR) function of sessile macrophages. Although a defect in reticuloendothelial system FcR-specific clearance has also been reported in SS [4], polymorphonuclear leukocytes (PMN) have not been examined in this respect. It was therefore of interest to study phagocyte function on isolated PMN in a patient with primary SS.

Materials and Methods

Patient: A 50-year-old woman entered hospital because of an extensive postthrombotic ulceration of her right lower leg. Her extremities exhibited sclerodermiform changes. Around her mouth there was radial furrowing. Her hair was sparse and brittle, diffuse alopecia involved her scalp. Her nose and cheek exhibited erythema. Her voice appeared hoarse. Keratoconjunctivitis sicca, xerostomia, dysphagia, dyspnoea had existed for about 12 years. She appeared cachectic weighing 44 kg, being 164 cm tall. Her liver was enlarged to 16 cm in the medioclavicular line.

On laboratory examination she exhibited polyclonal hypergammaglobulinemia of about 50% with a reduction of albumin to 30%. Serum IgG was elevated to 3,927 mg/dl and IgA to 612 mg/dl, while the IgM value was 52 mg/dl. The erythrocyte sedimentation rate was 94 mm during the 1st. h. She had thrombocytosis of 502/μl and a slight reduction of serum hemoglobin to 11.7 g/dl. The serum iron was diminished to 15 μg/dl. Tests for antinuclear antibodies were positive with a titer of 1:160 of the homogeneous/speckled type. Fur-
thermore, she was positive for Ro/SSA and La/SSB antibodies. The Raji cell test demonstrated circulating immune complexes. Skin testing with recall antigens (Multitest Mérieux) exhibited diminished responsiveness with a score of 3 mm (norm > 5). Her HLA phenotype was A1, 2; B8, w60; Cw3, w7/2.

Spleen function testing with heat-altered, $^{99m}$Tc-labeled autologous erythrocytes exhibited a prolonged splenic clearance value of 9.0 min (norm 3.5–5.5 min); her spleen size was normal. Roentgenograms of the chest showed interstitial fibrosis. Esophageal manometry exhibited markedly diminished peristalsis. Ophthalmologic examination yielded minimal lacrimal secretion as ascertained by the Schirmer test, filiform corneal ulcerations and scars consistent with keratoconjunctivitis sicca; a dental examination showed marked xerostomia. These findings established the diagnosis of primary SS.

**Control**: The control individual was a healthy 53-year-old woman whose HLA phenotype was A3, 32; B44, w62; Cw3, w5.

**PMN Function Tests**: Chemiluminescence as detailed [9] served to assess the respiratory burst in PMN. Dulbecco’s MEM (Boehringer, Mannheim) allowing pH stability was used as cell-suspension medium. The incubation was performed in a water bath at 37°C; every 10 min up to 60 min the chemiluminescent activity of duplicate samples was determined and expressed as the sum of the means of each set of values, i.e. integrated counts.

Stimuli for PMN included zymosan particles (Z; 0.33 mg/ml), opsonized zymosan (OpsZ; 0.33 mg/ml) acting as a C3b stimulant, zymosan-activated serum (ZAS; 4 vol. fresh normal human serum exposed to 1 vol. 0.33 mg/ml zymosan for 30 min at 37°C) defined as stimulating C5a receptors, as well as phorbol-myristate-acetate (PMA) [6]. Heat-aggregated (62°C, 10 min) bovine immunoglobulin (Behringwerke) served as FcR stimulus [8].

**Binding of Aggregated IgG to PMN**: Duplicate samples of 2.5 x 10⁶ PMN were incubated with 0.2 ml phosphate-buffered saline (PBS), 0.1% sodium azide (Merck), and 0.2 ml heat-aggregated immunoglobulin (50 mg/ml) donated by Biotest (Dreieich, FRG) for 30 min at 37°C. After washing with 0.1% azide-buffer, 5 μl $^{125}$I-labeled F(ab')₂ fragment (spec. activity 18 μCi/μg; 50 μCi/0.5 ml; Amersham) of anti-human Ig from sheep were incubated with the PMN in a final volume of 0.4 ml for another 30 min at 37°C and washed thrice. Radioactivity was determined in a gamma counter. Binding of $^{125}$I-labeled F(ab')₂ fragments alone to the PMN was minimal and could be neglected (data not shown).

**Results**

**Functional FcR Studies in PMN**: Upon stimulation with aggIg, Fig. 1 shows control PMN to respond with a dose-dependent stimulation of the respiratory burst, contrasting the PMN of the patient with SS. To stimulation with Z, OpsZ, ZAS, and PMA, however, the patient responded similarly to the control. In the case of ZAS, the patient’s response was even 186% higher than that of the control with regard to basal activity. Likewise, the activity of unstimulated PMN was 176% higher in the patient.

When fresh sera of the patient, control, and another healthy individual were incubated with PMN of patient and control, both in the presence

![Fig. 1. Chemiluminescent response of 5 x 10⁵ isolated PMN from the patient with Sjögren’s syndrome (■—■) and the control (○—○) upon stimulation with aggIg at 37°C. The chemiluminescence of duplicate samples was determined every 10 min up to 60 min. The ordinate displays the sums of the means. For comparison, stimulation by zymosan was 1,222 and 1,064, by opsonized zymosan 1,375 and 1,349, by zymosan-activated serum (1/6 dilution) 136 and 135, by phorbol-myristate-acetate 1,311 and 1,068 (x 10³) counts in the patient vs the control, respectively.](image-url)