ENHANCEMENT BY IMMUNOGLOBULIN M OF OXYGEN CONSUMPTION OF LEUKOCYTES DURING PHAGOCYTOSIS

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1. Introduction

The serum components known to facilitate phagocytosis include immune antibodies, natural antibodies and complements [1]. Rowley and Turner [2] found that immunoglobulin M obtained from immunized rabbit serum stimulates phagocytosis of leukocytes which bound to particles and the stimulating effect of immunoglobulin M is greater than that of immunoglobulin G. Smith et al. [3] reported that immunoglobulin G is more effective on phagocytosis than immunoglobulin M. On the other hand, Rowley and Jenkin [4] and Svehag [5] pointed out the importance of a natural antibody for phagocytosis and they isolated a phagocytosis-promotive protein fraction from normal serum which is mainly 19S immunoglobulin.

Under these circumstances, we isolated immunoglobulin M from normal bovine serum in a pure form and observed the stimulating effect of immunoglobulin M on oxygen consumption of bovine leukocytes during phagocytosis in the presence of *Micrococcus lysodeikticus*.

2. Materials and methods

Bovine leukocytes were isolated by the method described previously [6] and suspended in the buffered saline at pH 7.4 [7] to give a concentration of 5×10^8 cells/ml. Immunoglobulin M was purified from bovine serum by the method of Cambier and Butler [8] with a slight modification. Bovine serum (700 ml) was dialyzed against 20 liters of 0.01 M potassium phosphate buffer (pH 5.4) for 24 h at 4°C. Euglobulin precipitates were washed twice with the dialysis buffer and dissolved in 120 ml of 0.01 M acetate buffer (pH 5.4) containing 0.15 M NaCl. After removing unsoluble proteins by centrifugation, 0.1 M $ZnSO_4$ (40 ml) was added dropwise to the supernatant at 25°C with constant stirring. The precipitates were removed by centrifugation. To the sample solution was added Na_2EDTA (1% w/v), which chelates excess zinc ions. The sample protein solution was subjected to chromatography with Sepharose 4B column (3.5 × 55 cm) equilibrated with 0.1 M Tris–HCl buffer containing 1 M NaCl (pH 8.6). The fractions eluted from the first protein peak were identified as immunoglobulin M by analyzing 3% acrylamide gel electrophoresis and immunoelectrophoresis.

Phagocytic activity of leukocytes was determined by measuring the fluorescence intensity of dansylated *M. lysodeikticus* which bound to or was engulfed by leukocytes and also by measuring O_2 consumption of leukocytes in the presence of *M. lysodeikticus*. *M. lysodeikticus* cells were heat-killed and washed with buffered saline. Dansylation of *M. lysodeikticus* cells was done by adding dansyl chloride (20 mM) to bacterial cells in isotonic bicarbonate buffer (pH 9.0) at 37°C for 1 h and dansylated bacterial cells were washed with buffered saline. To a protein solution (3.5 ml)† containing 3×10^{-3} M Ca^{2+} and 3×10^{-4} M

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† A protein solution was obtained by dialyzing each fractionated protein solution against buffered saline (pH 7.4).

Mg^{2+} was added 50 μl of leukocyte suspension followed by 50 μl of dansylated bacterial suspension (5×10^{10} cells/ml). After incubation for 5 min at 37°C, phagocytosis was stopped by rapidly cooling the reaction mixture. Leukocytes bound to dansylated cells, which are free from unphagocytosed bacterial cells, were obtained by centrifugation at low speed and dissolved by adding 1.5% sodium dodecylsulfate, and then the fluorescence intensity was measured at 510 nm (excitation wavelength: 350 nm). Measurement of oxygen consumption of leukocytes was carried out with a closed plastic chamber equipped with a Clark oxygen electrode at 37°C with constant stirring. The mixture of a protein solution (3.5 ml)* containing 3×10^{-3} M Ca^{2+} and 3×10^{-4} M Mg^{2+} and leukocyte suspension (50 μl) was put into the chamber and 50 μl of bacterial suspension was added to the leukocyte suspension. The oxygen consumption was recorded with time.

3. Results and discussion

Fig.1 shows a chromatogram of serum proteins with Sepharose 4B column mentioned above (curve A) and the rate of oxygen consumption of leukocytes in each fractionated protein solution in the presence of *M. lysodeikticus* (curve B). In curve A, two major protein peaks exist in which the first peak is immunoglobulin M and the second peak seems to the immunoglobulin G. The pattern of the first protein fraction in curve A fairly agrees with that of the rate of oxygen consumption of leukocytes in each protein fraction (curve B), which indicates that immunoglobulin M enhances oxygen consumption of leukocytes in the presence of bacterial cells. Stimulating effect of immunoglobulin G on phagocytosis was scarcely observed.

* See footnote † preceding page.

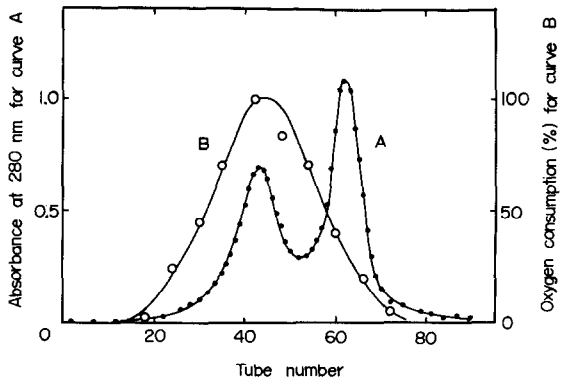

Fig.1. Chromatogram of serum proteins, obtained by the addition of $ZnSO_4$ and of EDTA to euglobulin fraction [8], with Sepharose 4B (3.5 × 55 cm) equilibrated with 0.1 M Tris–HCl buffer (pH 8.6) containing 1 M NaCl, 37°C pH 7.4. Curve A: protein; Curve B: relative rate of oxygen consumption of leukocytes in the presence of *M. lysodeikticus*.

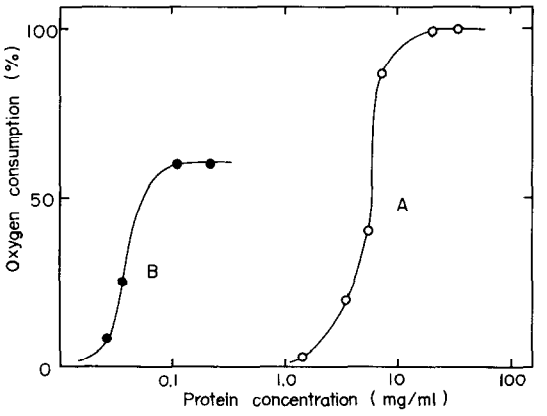

Fig.2. Plotting of oxygen consumption of leukocytes in the presence of bacterial cells against protein concentration. Curve A: serum; Curve B: immunoglobulin M, 37°C, pH 7.4.

Fig.2 represents the plotting of oxygen consumption of leukocytes against protein concentration, in which curves A and B are the results obtained for serum and immunoglobulin M, respectively. As is clear from these curves, the half value of the maximum oxygen consumption in the sigmoidal curve, C_m, is 6 mg protein/ml for serum (curve A), which is approximately 150 times greater than C_m = 0.04 mg protein/ml for immunoglobulin M (curve B). The value of the maximum activity of oxygen consumption obtained for serum is 1.7 times greater than that for immunoglobulin M. The results indicate that immunoglobulin M enhances strongly the oxygen consumption of leukocytes in the presence of bacterial cells and that serum components except immunoglobulin M are necessary for exhibiting the full activity of oxygen consumption of leukocytes.

In order to clarify whether bacterial cells attach to and were engulfed by leukocytes or not, the degree of phagocytosis was tested by measuring the fluor-

Table 1
Fluorescence intensity (I_f) of dansylated bacterial cells phagocytosed by leukocytes.

Medium	I_f (%)
Buffered saline + Ca^{2+}, Mg^{2+}	0.07 (3)
Serum (70 mg protein/ml)	2.85 (100)
Dialyzed serum + Ca^{2+}, Mg^{2+}	2.90 (102)
Immunoglobulin M (1.2 mg protein/ml)	2.57 (90)

escence intensity of dansylated bacteria bound to leukocytes, and the results was summarized in table 1. Attachment or binding of dansylated cells to leukocytes was scarcely observed in buffered saline containing Ca^{2+} and Mg^{2+} ions. Addition of immunoglobulin M (1.2 mg protein/ml) to the buffered saline containing Mg^{2+} and Ca^{2+} ions gives rise to increment of fluorescence intensity which reflects the binding of a large number of dansylated cells to leukocytes. This phagocytic activity was the same degree as that in the presence of serum (70 mg protein/ml).

Experiments are in progress to clarify the mechanism of phagocytosis using a simple system containing leukocytes and bacterial cells in buffered saline with immunoglobulin M (1 mg protein/ml) instead of serum (70 mg protein/ml).

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