Fc-RECEPTOR MODULATES THE FUSION BETWEEN PHAGOCYTIC VESICLES AND LYSOSOMES IN GUINEA PIG POLYMORPHONUCLEAR LEUKOCYTES

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1. Introduction

Polymorphonuclear leukocytes and macrophages are facultative phagocytes, actively phagocytizing many foreign materials [1]. They have receptors for the active fragment of complement (C3b) and the Fc portion of immunoglobulin on their cell surface, and these receptors promote phagocytosis of particles opsonized with complement or antibody [1,2].

Little is known, however, about the role of these receptors in the fusion between phagocytic vesicles and lysosomes after phagocytosis. Under certain pathological conditions, some kinds of parasites have been reported to enter phagosomes of macrophages that are unable to fuse with lysosomes. If opsonized with antibody, these parasites become well phagocytized and also well transferred into phagosomes which, in turn, fuse with lysosomes [3]. Thus opsonization of the parasites seems to stimulate the fusion between the phagosomes and lysosomes, but the precise mechanism of this change is unknown.

This paper reports kinetical studies showing that the Fc-receptors of PMNs promote phagocytosis and also the subsequent fusion between phagocytic vesicles and lysosomes in the early stage of phagocytosis. This promotion of the fusion was not seen during reincubation of the cells after removal of the phagocytizable particles. Some kinds of the possible change in the fusing activity of the phagocytic vesicles that seemed to be caused by Fc-receptors are discussed in relation to phagocytic stimuli.

Abbreviations: PMN, polymorphonuclear leukocytes; POE, paraffin oil droplets; BSA, bovine serum albumin; IgG, immunoglobulin G; $F(ab')_2$, $F(ab')_2$ fragment of immunoglobulin G, digested with pepsin; Fc, Fc portion of immunoglobulin G; SDS, sodium dodecyl sulfate

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2. Materials and methods

2.1. *Materials*

Antibody against BSA was induced in rabbits with Freund's complete adjuvant (Difco, Detroit, MI). IgG was purified from sera as in [4]. The Fc portion of this IgG was digested with pepsin (Worthington, Freehold, NJ) and the $F(ab')_2$ portion was purified by Sephadex G-150 column chromatography as in [5]. Purified IgG and $F(ab')_2$ gave single bands of 150 000 and 95 000 mol. wt, respectively on SDS–polyacrylamide gel electrophoresis, and formed single precipitate lines which fused with each other on microplates in the Ouchterlony test. Other chemicals were obtained as in [6].

2.2. *Methods*

Paraffin oil emulsion coated with BSA (POE–BSA) was prepared as in [6]. Opsonization of POE–BSA with antibody was achieved by incubation of 0.65 mg IgG/ml with POE–BSA for POE–BSA–IgG, and 0.41 mg $F(ab')_2$/ml with POE–BSA for POE–BSA–$F(ab')_2$ at 37°C for 15 min just before use. At the concentrations used, neither IgG nor $F(ab')_2$ of the antibody agglutinated POE–BSA and the molar ratio of IgG to $F(ab')_2$ was 1:1.

Phagocytosis and isolation of the phagolysosome fraction were done as in [6]. The PMN were reincu-

bated at 37°C for 5 min after phagocytosis as in [6]. Removal of the cell surface POE after phagocytosis was checked under a light microscope. Assays were done as in [6].

3. Results

3.1. *Phagocytosis of POE*

As shown in fig.1, PMN phagocytized POE–BSA and POE–BSA–F(ab')$_2$ at about the same rate. The rate of uptake of POE–BSA–IgG was ~3 times that of POE–BSA in the first 5 min and then gradually decreased so that there was no remarkable difference between the uptakes in the 3 groups after 20 min. These results show that, unlike F(ab')$_2$ or normal rabbit IgG (data not shown), IgG against BSA had an opsonizing effect on phagocytosis of POE–BSA during the early part of incubation, that seemed to be attributable to the specific binding between Fc-receptors of PMN and POE–BSA–IgG.

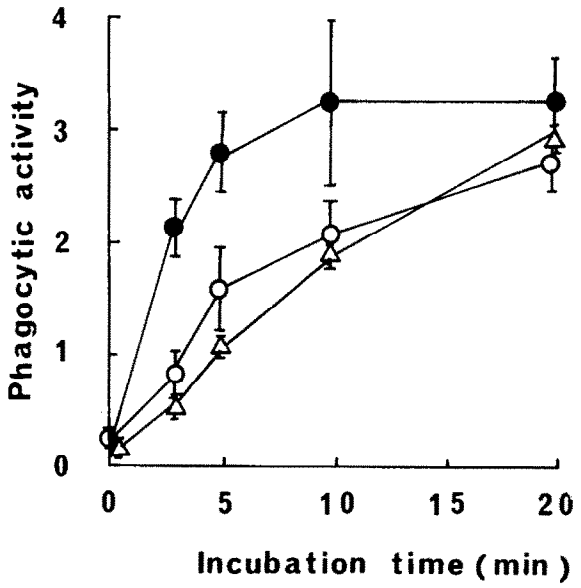

Fig.1. Time course of phagocytosis of POE–BSA–IgG (•——•), POE–BSA–F(ab')$_2$ (○——○) and POE–BSA (△——△). The number of POE was far in excess of the number of cells of this experiment. Phagocytic activity was calculated as nmol oil red O/mg cell protein in the cell homogenate. Values are shown relative to that of POE–BSA after incubation for 5 min (=1.00). Values are means ± SE for ≧4 expt.

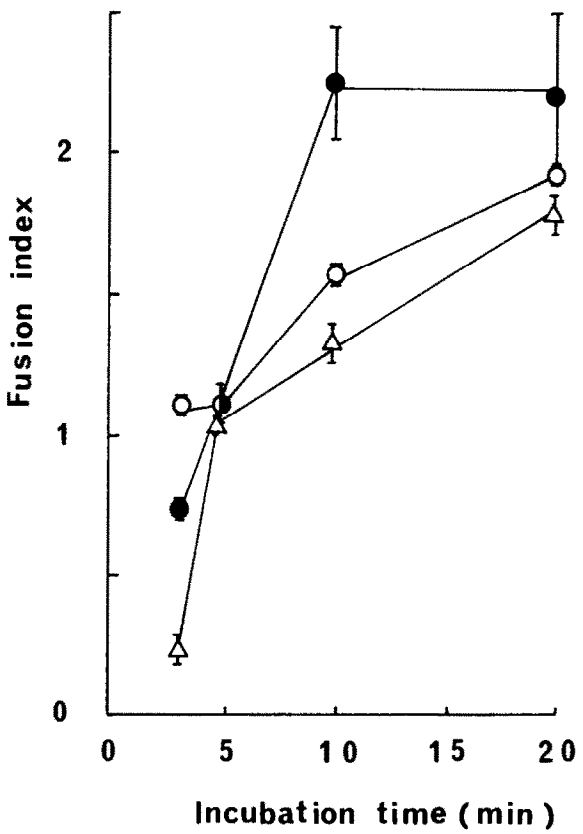

Fig.2. Time course of fusion during phagocytosis of POE–BSA–IgG (•——•), POE–BSA–F(ab')$_2$ (○——○) and POE–BSA (△——△). The fusion indices were calculated for acid phosphatase as described in the text, and are expressed relative to that of POE–BSA after 5 min, as in fig.1. Values are means ± SE for ≧3 expt.

3.2. *Effect of opsonization on the fusion between phagocytic vesicles and lysosomes during phagocytosis*

Extent of the fusion between phagocytic vesicles and lysosomes was expressed as the fusion index, which is defined in [6] and indicates the average extent of fusion with lysosomes per phagocytic vesicle.

As shown in fig.2, the fusion index reached nearly the same level in the 3 groups in 5 min, and then that with POE–BSA–IgG increased very rapidly within 10 min reaching a plateau, while those with POE–BSA and POE–BSA–F(ab')$_2$ increased rather slowly and gradually for the next 15 min. After 3 min, the level with POE–BSA–F(ab')$_2$ was highest, but this was only transient. These results seem to indicate

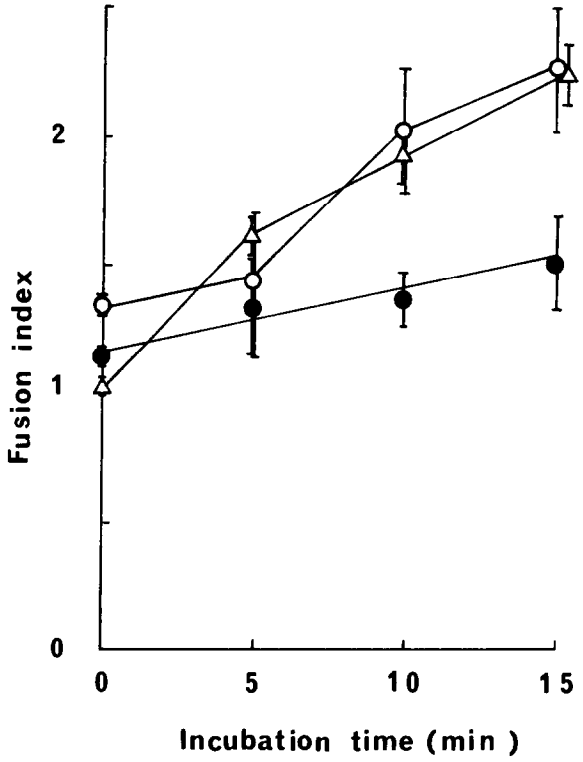

Fig.3. Time course of fusion without phagocytic stimulation. PMN phagocytized with POE–BSA–IgG (●——●), POE–BSA–F(ab')$_2$ (○——○) and POE–BSA (△——△) were reincubated in the same buffer as for phagocytosis, but without any phagocytizable particles at 37°C. No change of the fusion indices was observed on reincubation at 0°C (data not shown). The fusion indices were calculated and relative values are shown as in fig.3. Values are means ± SE for ≧4 expt.

that opsonized particles (POE–BSA–IgG) stimulated the fusion with lysosomes per phagocytic vesicle at an early stage of phagocytosis. This stimulation should be attributable to the function of Fc-receptors of PMN. No exocytosis of lysosomal enzymes was observed under the conditions used (data not shown).

3.3. *Fusion between phagocytic vesicles and lysosomes without phagocytic stimuli*

The results are shown in fig.3. Contrary to the results in fig.2, the fusion index of POE–BSA–IgG increased very slowly and linearly, while those of the other two increased at nearly the same rate and linearly, but slightly faster than that of POE–BSA–IgG. The results in fig.3, show that the rate and extent of fusion with lysosomes per phagocytic vesicle of POE–BSA–IgG was rather low without phagocytizable particles during reincubation, but the rates and extents per phagocytic vesicle of POE–BSA–F(ab')$_2$ and POE–BSA were not apparently different with or without phagocytizable particles. The results also suggest that opsonization rendered the phagocytic vesicles unable to fuse with lysosomes in PMN without phagocytic stimuli.

4. Discussion

Here, we have shown that POE–BSA–IgG, opsonized with the antibody, was phagocytized promptly and rapidly by PMN in the early period of incubation. F(ab')$_2$ of the antibody did not facilitate phagocytosis, suggesting that this effect of opsonization was Fc-receptor-dependent. Though various considerations should be taken into account in the use of xenogeneic rabbit IgG on guinea pig PMN, this IgG proved to be very useful in this work in investigations on Fc-receptor-dependent phagocytosis in PMN.

During phagocytosis, opsonization of POE–BSA with the antibody resulted in increase of the fusion index (fig.2). Thus the opsonization seems to facilitate also the fusion of phagocytic vesicles with lysosomes per phagocytic vesicle during phagocytosis. Conceivably Fc-receptors of PMN were internalized with POE–BSA–IgG through phagocytosis, and the resultant phagocytic vesicles with these receptors may have changed the characters more susceptible to fusion with lysosomes during phagocytosis (fig.2).

After phagocytizable particles were removed from the cell surface, the vesicles of POE–BSA–IgG became far less susceptible to fusion with lysosome, but vesicles of POE–BSA and POE–BSA–F(ab')$_2$ did not (fig.3). So, phagocytic stimuli seem to be necessary for fusion of lysosomes with the vesicles of POE–BSA–IgG, but not with other two types of vesicle. Binding products of Fc-receptors and the immune complex, whether within or without the surface, seem to modulate the fusing activity of the vesicles with lysosomes.

When phagocytic stimuli were removed, the Fc-receptors and immune complex of POE–BSA–IgG seem to result in lowering of the rate of fusion (fig.3). However, when phagocytic stimuli continued, the rate and extent of fusion of the phagocytic vesicles of POE–BSA–IgG were far higher than that without phagocytic stimuli (fig.2,3), though phago-

cytosis itself decreased and ceased after 5 min (fig.1). These findings suggest that the binding of Fc-receptors and the immune complex of POE–BSA–IgG stimulate fusion of the phagocytic vesicles with lysosomes from outside the cells and that this stimulation is necessary for fusion of the vesicles of POE–BSA–IgG.

Intracellular killing of opsonized-ingested bacteria by monocytes required binding of heterogeneous IgG to Fc-receptors of the cells [7], supporting the present findings. Though their report did not mention the fusion of phagocytic vesicles with lysosomes, our experiments suggest a relation between Fc-receptors and fusion. Further studies should clarify the mechanisms regulating fusion with lysosomes in relation to intravesicular and cell surface Fc-receptors.

Using POE–BSA [6] we found that fusion between phagocytic vesicles and lysosomes occurred without phagocytic stimuli or extracellular IgG, and this was confirmed here. Thus there seem to be at least two types of phagocytic vesicles with respect to fusion with lysosomes in PMN; one type (e.g., POE–BSA–IgG) is closely related with phagocytic stimuli and extracellular immune complex, whereas another type (e.g., POE–BSA and POE–BSA–(Fa'_2) is not. Studies are now in progress to elucidate the factors regulating fusion, and especially the roles of cell surface receptors [6], cytoplasmic movement of contractile proteins [6,8] and cellular metabolism [6].

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