CONCANAVALIN A RECEPTOR-MEDIATED PHAGOCYTOSIS AND SUBSEQUENT FUSION OF VESICLES WITH LYSOSOMES IN GUINEA PIG POLYMORPHONUCLEAR LEUKOCYTES

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1. Introduction

Some cell surface receptors have been reported to modulate cellular phagocytic activity [1]. Concanavalin A (con A) receptors have been especially extensively investigated in this respect and found to inhibit [2], to enhance [3] or to have no relation to [4] the rate and extent of phagocytosis, so their effect seems uncertain.

Studies on the effect of con A on fusion of phagocytic vesicles with lysosomes in mouse macrophages have also given conflicting results: One group found that it prevented phagolysosome formation [5], while another found that it did not [6].

Therefore, we studied the effect of con A on both phagocytosis and the subsequent fusion of phagocytic vesicles with lysosomes in guinea pig polymorphonuclear leukocytes (PMN). This paper describes our experimental system for allowing PMN to engulf con A-coated paraffin oil emulsion (POE–con A) via con A receptors, showing that con A stimulates phagocytosis and modulates the fusion with lysosomes during and after phagocytosis.

2. Materials and methods

2.1. *Materials*

Con A was either purchased from Miles-Yeda Lab. (Elkhalt, IN) or E. Y. Lab. (San Mates, CA) or was purified from Jack bean meal (Sigma, St Louis, MO) by the method in [7]. α-Methylmannoside (αMeMan) was obtained from Sigma (St Louis, MO). Other chemicals were obtained as in [8], and were of the purest commercial grade available.

Abbreviations: αMeMan, α-methyl-D-mannoside; BSA, bovine serum albumin; con A, concanavalin A; KRp, Krebs-Ringer phosphate buffer; PMN, polymorphonuclear leukocytes; POE, paraffin oil emulsion

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2.2. *Phagocytosis*

The conditions for phagocytosis of paraffin oil emulsion (POE) by PMN were as in [8]. POE was prepared from 1 vol. of paraffin oil, which had been stained with oil red 0, and 3 vol. 20 mg/ml of either con A or BSA in Krebs-Ringer phosphate buffer (with 1/3rd normal [Ca^{2+}]), pH 7.4 (KRp), as in [8]. Unbound con A and BSA were removed from POE by centrifugation, and stable POE, formed by repeated sonication, was used for phagocytosis as POE–con A and POE–BSA, respectively. Phagocytosis was started by adding 1/10 vol. POE to 1 vol. cell suspension in KRp prewarmed at 37°C, and continued for 0–20 min at 37°C, with shaking at 100 strokes/min. In some cases, 0.1 M αMeMan was added to the cell suspension during phagocytosis of POE–con A. Phagocytosis was stopped by adding ice-cold saline to the cells with POE–BSA, and 0.1 M αMeMan in ice-cold saline to those with POE–con A and rapidly cooling the mixture on ice. The cells were then washed extensively with saline and with 0.1 M αMeMan in saline, respectively, to remove non-ingested POE from the cell surface.

2.3. *Reincubation of PMN after phagocytosis and isolation of the phagolysosome fraction*

To investigate fusion between phagocytic vesicles and lysosomes in PMN without phagocytic stimuli, we reincubated the cells in KRp at 37°C or 0°C after

phagocytosis of POE at 37°C for 5 min as in [8].

The phagolysosome fraction was isolated from the cells just after phagocytosis and after reincubation as in [8].

2.4. *Assays*

The activity of lysosomal acid phosphatase, and the concentrations of oil red 0 in POE and of protein were determined as in [8]. Alkaline phosphatase which is a granular enzyme but not a lysosomal enzyme, was assayed with *p*-nitrophenylphosphate at pH 10.0 [9].

3. Results

3.1. *Phagocytosis of POE*

As shown in fig.1, POE–con A was taken up by PMN far more rapidly than POE–BSA, which was used as a control for POE–con A because it is not phagocytosed via specific receptors. Addition of 0.1 M αMeMan with POE–con A did not inhibit the uptake of POE–con A appreciably and decreased the rate and extent of phagocytosis only slightly. When 0.1 mg/ml of soluble con A was incubated with PMN at 0°C for 30 min before phagocytosis of POE–con A, the level of phagocytosis in 5 min at 37°C decreased to 13% of that of untreated cells. This decrease was completely prevented by addition of 0.1 M αMeMan during preincubation with soluble con A (not shown). These results indicate that POE–con A is phagocytosed via con A receptors on the surface of PMNs.

A few droplets of POE–con A were sometimes seen attached on the cell surface after extensive washing with 0.1 M αMeMan in saline, as shown by the values at 0 time in fig.1. The number of these POE–con A droplets attached to the cell surface remained unchanged during phagocytosis for 0–20 min, and was much less than the number of POE–con A droplets internalized. POE–BSA was mostly internalized and was very rarely seen attached to the cell surface.

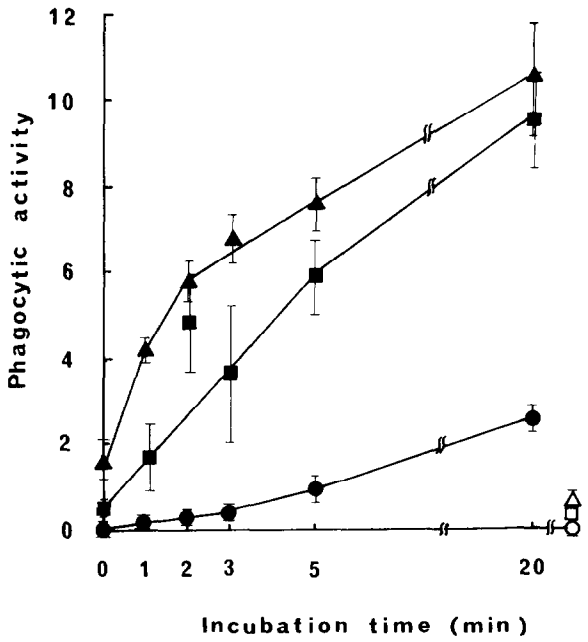

Fig.1. Time course of phagocytosis of POE–con A (▲), POE–con A + αMeMan (■) and POE–BSA (●). Phagocytic activity was calculated as nmol oil red 0/mg cell protein in the homogenates and is shown relative to the phagocytosis of POE–BSA after 5 min. Corresponding open symbols (△,□,○) indicate the levels of attachment in 30 min at 0°C. Values are means ± SE for 3 expt.

3.2. *Fusion of phagocytic vesicles with lysosomes during phagocytosis*

The extent of fusion between phagocytic vesicles and lysosomes was measured in two ways:

(i) As the % recovery of lysosomal enzymes in the phagolysosome fraction [10], which represents the extent of conversion of the lysosomal compartment from primary (unfused) lysosomes to secondary (phago-)lysosomes.

(ii) As the fusion index [8], which represents the extent of fusion with lysosomes/phagocytic vesicle.

The results in fig.2 indicate that the amounts of both acid and alkaline phosphatases recovered in the phagolysosome fraction increased as phagocytosis proceeded (fig.1), and the difference between the results with POE–BSA and POE–con A (±αMeMan) was greater for alkaline phosphatase than for acid phosphatase. Values for the % recovery varied in different experiments in parallel with differences in phagocytic activity. Results on the other indicator, the fusion index, are shown in table 1. With POE–BSA the fusion indices of acid and alkaline phosphatases increased; with POE–con A they did not change significantly; with POE–con A + αMeMan the index of alkaline phosphatase did not change appreciably, as with POE–con A, but that of acid phosphatase increased.

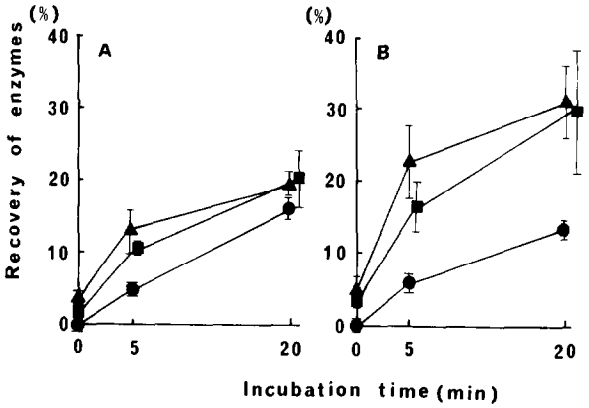

Fig.2. The % recovery of acid (A) and alkaline (B) phosphatases in phagolysosome fractions during phagocytosis. The recovery was calculated as follows:

$$\text{Recovery (\%)} = \frac{\text{Enzyme in the phagolysosome fraction}}{\text{Enzyme in the homogenate}} \times \frac{\text{Oil red O in the homogenate}}{\text{Oil red O in the phagolysosome fraction}} \times 100$$

Values are means ± SE for 2 typical expt. The results of other experiments showed similar patterns but with greater deviations. The symbols are the same as in fig.1 for POE–con A (▲), POE–con A + αMeMan (■) and POE–BSA (●).

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

As shown in fig.3, the fusion indices did not change at 0°C but increased to various extents at 37°C. The fusion indices of acid phosphatase in the three groups increased to the same extent for 5 min; then with POE–BSA it increased further, while with POE–con A it remained constant. The indices of alkaline phosphatase showed the same change as

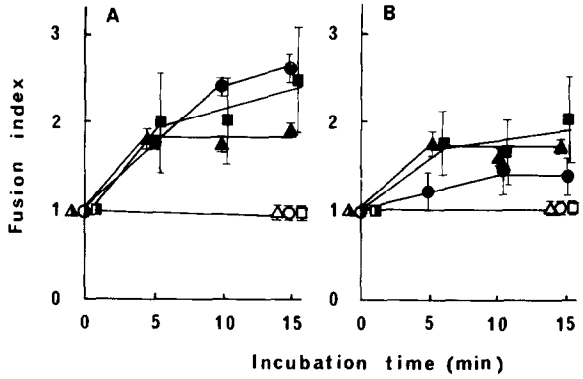

Fig.3. Time course of fusion during reincubation without phagocytic stimulation. Fusion indices were calculated as described in the text for acid (A) and alkaline (B) phosphatases and are shown relative to the value at 0 time in each group. Values are means ± SE for 3 expt. The symbols are the same as in fig.1 for POE–con A (△,▲), POE–con A + αMeMan (□,■) and POE–BSA (○,●). Closed symbols show the results of incubation at 37°C, and open symbols those at 0°C.

those of acid phosphatase with POE–con A, but were considerably lower with POE–BSA. With POE–con A + αMeMan the indices of the two enzymes showed similar patterns to those with POE–con A, but with larger deviations.

4. Discussion

Here we have developed an experimental system for investigating the effect of con A on both phagocytosis and subsequent fusion with lysosomes using POE–con A as phagocytizable particles. These particles seem to be phagocytized via con A receptors, as described, though 0.1 M αMeMan did not inhibit phagocytosis of POE–con A effectively.

Table 1
Changes of the fusion index during phagocytosis

Phagocytosis of	Acid phosphatase		Alkaline phosphatase	
	5 min	20 min	5 min	20 min
POE–BSA	1.00	1.73 ± 0.33	1.00	1.74 ± 0.03
POE–con A	1.00	1.06 ± 0.11	1.00	1.21 ± 0.02
POE–con A + αMeMan	1.00	1.63 ± 0.32	1.00	1.13 ± 0.17

Fusion indices were calculated as in the text and are shown relative to the value after 5 min in each group. Values are means ± SE for the same 2 expt as in fig.2

Mode of interaction of cells with soluble con A may be different from that with POE–con A, on which con A is greatly concentrated. Conceivably POE–con A particles cause the accumulation of con A receptors to the loci of attachment on the cell surface, resulting in invagination and formation of phagocytic vesicles rapidly and actively enough to resist the attack of αMeMan on the con A. So, this system differs from other systems of phagocytosis with soluble con A [2–6]. But whatever the mechanism involved, POE–con A were phagocytized far more rapidly than POE–BSA, and since POE–con A and POE–BSA resemble each other in shape, structure, size variation and stability in KRp, this finding shows that con A facilitates phagocytosis of POE.

During phagocytosis, phagocytic vesicles of POE–con A fused with lysosomes (acid phosphatase) and with specific granules (alkaline phosphatase) [9,11], as in the case of phagocytosis of bacteria [12], and the recovery of enzymes increased with time (fig.2). The extent of fusion/phagocytic vesicle of POE–con A remained constant during phagocytosis (table 1), and it increased ~1.8-times and reached a plateau during reincubation without the phagocytic stimuli from outside for 5 min (fig.3). So, phagocytic stimuli seem to modulate the fusion of the vesicles of POE–con A. Soluble con A has been shown to enhance fusion of the vesicles of POE–BSA with lysosomes during reincubation [8], so at least, con A as POE–con A or soluble con A does not seem to inhibit phagolysosome formation in PMN. In the case of POE–BSA, the fusion indices of both enzymes increased ~1.7-fold during 5–20 min phagocytosis (table 1). But during reincubation after phagocytosis for 15 min the index of acid phosphatase increased more, and that of alkaline phosphatase increased less than with POE–con A (fig.3). These results suggest that phagocytic stimuli modulate the extent of fusion variously and that the extent of fusion of phagocytic vesicles varies with the kind of POE in the vesicles and also with the kind of cytoplasmic granules (lysosomes or specific granules).

Our experimental system seems to be a useful tool for use in studies on the role of con A receptors in phagocytosis and subsequent fusion of phagocytic vesicles with lysosomes and specific granules.

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