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MACROPHAGE HYDROGEN PEROXIDE PRODUCTION AND PHAGOCYTIC FUNCTION ARE DECREASED FOLLOWING PHAGOCYTOSIS MEDIATED BY Fc RECEPTORS BUT NOT COMPLEMENT RECEPTORS

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Previous in vivo and in vitro studies have shown that the phagocytosis of IgG-coated erythrocytes results in a depression of macrophage function. The present study compared the effect of phagocytosis mediated by Fc receptors with that mediated by complement receptors. The phagocytosis of IgG-coated erythrocytes by elicited peritoneal macrophages depressed their capacity to produce hydrogen peroxide as well as phagocytic function. Phagocytosis of erythrocytes coated with IgM and complement had neither of these effects. These results implicate the intracellular signaling that results from Fc receptor mediated phagocytosis in the depression of macrophage function that is caused by phagocytosis. © 1991 Academic Press, Inc.

Challenge of macrophages by the phagocytosis of IgG-coated erythrocytes (EIgG) in vitro has been shown to decrease macrophage phagocytic function, respiratory burst capacity and bactericidal activity (1-3). These effects probably contribute to the increase in mortality rate with bacterial infection that has been observed following the phagocytosis of EIgG by Kupffer cells in vivo (4,5). The mechanism for the depression of macrophage function following phagocytosis is unknown, but the intracellular signalling from the receptor mediating the phagocytosis could be important in causing this effect.

The present study compared the effect of a phagocytic challenge mediated by Fc receptors with a challenge mediated by complement receptors. It was found that macrophage H_2O_2 production and phagocytic function were depressed following Fc receptor-mediated phagocytosis but not with complement receptor-mediated phagocytosis. This finding implicates the intracellular signaling that results from Fc receptor-mediated phagocytosis in the depression of macrophage function that is caused by a phagocytic challenge.

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METHODS

Macrophages. Rat peritoneal macrophages elicited with casein were isolated as previously described (1). Briefly, macrophages were obtained by peritoneal lavage of Sprague-Dawley rats (250-275 g). The exudate cells were suspended in Dulbecco's modified Eagle's media (DMEM) containing 100 units/ml of penicillin and 100 µg/ml of streptomycin and plated in 24-well tissue culture plates at a density of $2x10^6$/well. Non-adherent cells were removed by washing after incubation for 2 hr at 37°C in 5% CO_2 in air and after incubation overnight in DMEM containing 10% bovine calf serum. The resulting monolayers contained about $6x10^5$ cells/well and were 95% macrophages as determined by Wright-stained morphology.

Phagocytic challenge. Macrophages were challenged with phagocytosis mediated by Fc or complement receptors. Fc receptor-mediated phagocytosis was induced by incubating the macrophages with sheep erythrocytes coated with anti-erythrocyte IgG (EIgG), and complement receptor-mediated phagocytosis was induced with erythrocytes coated with anti-erythrocyte IgM (EIgM) that were incubated in serum (EIgMC). EIgG and EIgM were prepared using the procedure previously described for the preparation of EIgG (1). EIgMC were prepared by incubating EIgM in 10% mouse serum at 37°C for 30 min. The EIgMC were washed twice and used within 2 hrs. Mouse serum was obtained from Swiss-Webster mice and stored at -70°C.

Macrophages were incubated with EIgG or EIgMC for 1 hr to allow phagocytosis. Controls were incubated with an equal number of unopsonized erythrocytes. Then the macrophages were washed to remove non-adherent erythrocytes and the adherent, non-internalized erythrocytes were hypotonically lysed. Following this phagocytic challenge, macrophages were incubated for 1 hr before determining H_2O_2 production or Fc receptor phagocytic function. The phagocytosis of EIgG and EIgMC was determined using erythrocytes labeled with ^{51}Cr, and the radioactivity associated with the macrophages after the hypotonic lysis was taken as the amount of phagocytosis.

Assay of H_2O_2 production. Macrophage H_2O_2 production, triggered by phorbol myristate acetate (PMA, 50 ng/ml), was determined as previously described (6).

Assay of Fc receptor phagocytic function. Macrophage Fc receptor phagocytic function was determined from the percent of a $1x10^7$ dose of EIgG that was phagocytized in 1 hr as previously described (1).

Statistics. All measurements were made on triplicate monolayers using at least 3 separate macrophage isolations. Data are expressed as the mean ± SEM. Comparisons were analyzed using a paired t-test or analysis of variance. The level of confidence is placed at 95% for all experiments.

RESULTS

A greater percentage of the challenge doses of EIgG were phagocytized than EIgMC. For challenge doses of $1x10^7$, $5x10^7$ and $1x10^8$, the number of EIgG phagocytized was 2.6±0.2, 8.9±0.5, and 22.4±1.9 x 10^6/well, respectively; and the number of EIgMC phagocytized was 1.0±0.2, 3.3±0.5, and 5.8±0.4 x 10^6/well, respectively. Macrophages

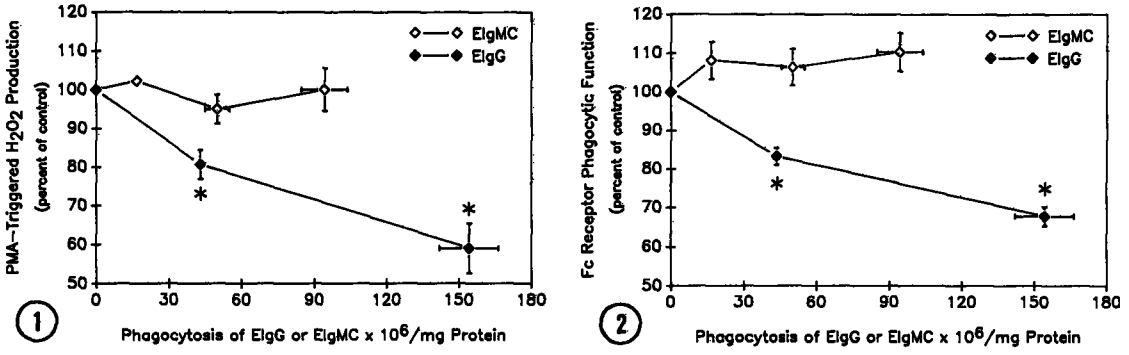

Figure 1. The effect of a phagocytic challenge with EIgG and EIgMC on PMA-triggered H_2O_2 production. Macrophages were challenged by adding EIgG or EIgMC to the wells and incubating for 1 hr to allow phagocytosis. The adherent non-internalized erythrocytes were then hypotonically lysed and the macrophages incubated for an additional hr before determining H_2O_2 production. The amount of phagocytosis that occurred during the phagocytic challenge was determined in separate wells at the end of the challenge period. Data are shown for challenge doses of EIgMC of $1x10^7$, $5x10^7$, and $1x10^8$ and for doses of EIgG of $1x10^7$ and $5x10^7$. H_2O_2 production values are expressed as percent of control and phagocytosis values indicate the number of EIgG or EIgMC phagocytized as a result of challenge with 1×10^7 and 5×10^7 EIgG/well and 1×10^7, 5×10^7, and 1×10^8 EIgMC/well. Values are the mean ± SEM for 4–5 experiments.
*$p < 0.05$.

Figure 2. The effect of a phagocytic challenge with EIgG and EIgMC on phagocytic function. The experiment was carried out as described in the legend for Figure 1, except that phagocytic function was determined. Phagocytic function values are expressed as percent of control and phagocytosis values indicate the number of EIgG or EIgMC phagocytized as a result of challenge with 1×10^7 and 5×10^7 EIgG/well and 1×10^7, 5×10^7, and 1×10^8 EIgMC/well. Values are the mean ± SEM for 5 experiments.
*$p < 0.05$.

that had phagocytized similar numbers of EIgG or EIgMC appeared identical under phase contrast microscopy. Phagocytosis of unopsonized erythrocytes was less than 3% of the dose.

H_2O_2 production triggered by PMA was decreased in a dose-dependent manner following EIgG phagocytosis (Fig. 1). With the 1×10^8 challenge dose the depression was 48.2±8.2 percent of control (data not shown). In contrast to the effect of EIgG phagocytosis, there was no change in H_2O_2 production following EIgMC phagocytosis (Fig. 1). Unopsonized erythrocytes had no effect of H_2O_2 production.

Changes in Fc receptor phagocytic function were similar to those observed for H_2O_2 production (Fig. 2). Phagocytic challenge with $1x10^8$ EIgG depressed phagocytic function to 53.3±4.3 percent of control (data not shown). Phagocytic challenge with EIgMC did not cause a change in phagocytic function (Fig. 2). While the amount of the phagocytic challenge taken up was less for each dose of EIgMC than for EIgG, comparison of the effect of the higher doses of EIgMC with the effect of the lower doses of EIgG shows that the phagocytosis of EIgMC depressed neither H_2O_2 production nor phagocytic function.

DISCUSSION

The depression of macrophage H_2O_2 production and phagocytic function following a phagocytic challenge with EIgG is consistent with our previous work (1). Our results with H_2O_2 production are similar to those reported by Stewart et al. (2) even though they used mouse peritoneal macrophages elicited by Corynebacterium parvum, determined H_2O_2 production at 3 hr after the phagocytic challenge and triggered H_2O_2 production with zymosan. Their phagocytic challenge was comparable to our $5x10^7$ dose of EIgG as was the 52% decrease in H_2O_2 production. The depression of macrophage function following EIgG phagocytosis suggests that the erythrocyte phagocytosis associated with thermal injury may contribute to the impairment of host defense against bacterial infection that is associated with this form of injury (4).

While Fc receptor–mediated phagocytosis of EIgG depressed macrophage function, the phagocytosis of EIgMC via complement receptors had no effect. Few previous studies have compared the effect of a phagocytic challenge mediated by Fc and complement receptors. Esparza et al. (7) demonstrated that the phagocytosis of either EIgG or EIgMC resulted in a depression of macrophage tumoricidal function. The macrophages had to be exposed to 3 times more EIgMC than EIgG before an effect was observed, but the amount of phagocytosis was not reported. Thus, tumoricidal function of macrophages can be inhibited by the phagocytosis of EIgMC, but the present study indicates that respiratory burst capacity and phagocytic function of less activated macrophages is not depressed.

It has been shown that the phagocytosis of IgG–coated erythrocyte ghosts does not depress macrophage function, suggesting that erythrocyte contents may be involved (1,2,5). We have shown that the scavengers of reactive oxygen intermediates within erythrocytes are not required for the depression of H_2O_2 production following EIgG phagocytosis (6). Stewart et al. (2) have shown that the ingestion of hemoglobin can depress macrophage H_2O_2 production. The present study indicates that erythrocyte contents are not sufficient to depress macrophage function, because the phagocytosis of EIgG depressed macrophage function but the phagocytosis of a similar number of EIgMC did not have this effect.

The observation that a phagocytic challenge with EIgG depressed macrophage function, but challenged with EIgMC did not, implicates Fc receptor signaling in the mechanism of the effect of phagocytosis. The effect of EIgG phagocytosis could be the result of an Fc receptor–mediated increase in intracellular cAMP levels or an effect on the release of arachidonic acid. Nitta and Suzuki (8) have shown that Fc receptor–mediated

phagocytosis is followed by an increase in intracellular cAMP levels. An increase in cAMP levels has been shown to be capable of depressing macrophage respiratory burst capacity and phagocytic function (9,10). In contrast to phagocytosis mediated by Fc receptors, complement receptor–mediated phagocytosis does not stimulate arachidonic acid release (11). Arachidonic acid and its metabolites are involved in the regulation of the respiratory burst (10). Henderson et al. (12) have shown that blocking phospholipase A_2 activity decreased macrophage superoxide production and that the addition of arachidonic acid restored the superoxide production. We have shown that the ability of macrophages to release arachidonic acid was depressed following EIgG phagocytosis and that the addition of arachidonic acid restored H_2O_2 production following EIgG phagocytosis (unpublished). Cycloxygenase blockers did not modify the effects of EIgG phagocytosis on H_2O_2 production. Whatever the mechanism for the depression of macrophage function following EIgG phagocytosis, the present study extends our understanding of this effect by implicating the intracellular signaling that results from Fc receptor–mediated phagocytosis in the development of impaired macrophage function.

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