DISSOCIATION OF PHAGOCYTOSIS FROM STIMULATION OF THE OXIDATIVE METABOLIC BURST IN MACROPHAGES

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Plasma membrane receptors for the Fc portion of IgG and for the major fragment of the third component of complement (C3b)[1] play an essential role in the function of macrophages as phagocytic cells. Fc receptors mediate both attachment and phagocytosis of particles coated with IgG antibody (1–3). Receptors for C3b on normal (resident) mouse peritoneal macrophages mediate only the attachment of particles to the cell (1, 3, 4), whereas C3b receptors on macrophages activated by infection or macrophages elicited by injection of inflammatory agents mediate both attachment and ingestion (3, 5–7).

During phagocytosis, or as a consequence of perturbation of the phagocyte plasma membrane by soluble stimuli such as phorbol myristate acetate (PMA), the cell undergoes a "respiratory burst," in which it consumes oxygen and converts it to the potent microbicidal agents superoxide anion (O_2^-), hydrogen peroxide, hydroxyl radical, and perhaps, singlet oxygen. The role of phagocytosis through Fc or C3b receptors in stimulation of this oxidative burst has not been defined in macrophages. Studies with neutrophils in this regard suggest that the C3b receptor permits the attachment of C3b-coated particles to the cell; however, additional interaction with a second receptor (e.g., Fc receptor [8, 9] or receptor for a component of fungal cell wall [9], perhaps conjugated mannose [10]) appears to be required for both phagocytosis and stimulation of the respiratory burst. In these systems, ingestion has invariably been associated with stimulation of oxidative metabolism. We report here that phagocytosis of IgG-coated sheep erythrocytes (E) by macrophages elicited a vigorous oxidative response. In contrast, phagocytosis by macrophages of E coated with IgM and complement or E treated with glutaraldehyde was not accompanied by stimulation of the respiratory burst. This dissociation could protect the host from oxidative tissue damage.

[1] *Abbreviations used in this paper:* BCG, bacille Calmette-Guérin; C3b, major fragment of the third component of complement, before or after cleavage by factor I; E, sheep erythrocyte; E(IgG), E coated with anti-E IgG; E(IgM), E coated with anti-E IgM; E(IgM)C, E coated with anti-E IgM and with the first four components of complement; GE, glutaraldehyde-treated E; KRPD, Krebs-Ringer phosphate buffer with 11 mM dextrose; LPS, lipopolysaccharide; PBS, phosphate-buffered saline; PMA, phorbol myristate acetate; RBC, red blood cells without regard to the presence or nature of attached Ig or complement.

Materials and Methods

Macrophages. Mouse peritoneal macrophages were harvested, washed, and suspended in medium supplemented with 20% heat-inactivated fetal calf serum (Sterile Systems, Inc., Logan, UT) (11); approximately 1×10^6 cells in a total volume of 1 ml were plated on 16-mm diam tissue culture dishes or 13-mm diam glass coverslips (12). After incubation for 120 min at 37°C in 5% CO_2-95% air, plated cells were washed vigorously with medium twice, then cultured overnight (11). The cells adherent after overnight culture were washed with Krebs-Ringer phosphate buffer with 11 mM dextrose (KRPD) before assay. Peritoneal macrophages were elicited with lipopolysaccharide (LPS) or obtained from bacille Calmette-Guérin (BCG)-infected mice as previously described (11).

With each type of peritoneal exudate, cells adherent after overnight culture were >97% macrophages and <1% granulocytes, as estimated by differential counts of stained cells (11) and by phagocytic capacity for candida (12). The protein content of the dishes at the time of assay was 15–40 µg/dish (11).

Erythrocytes. Antibody-coated or antibody plus complement-coated E were prepared as described by Bianco et al. (3) and Griffin et al. (4), with slight modification. IgG and IgM made from rabbit anti-E antiserum were obtained from Cappel Laboratories, Cochranville, PA. The IgG (33 mg/ml) agglutinated E at ditutions of up to 1:10, 240. Trace amounts of IgG were removed from the IgM preparation by adsorption with Sepharose CL 4B-protein A (Pharmacia Fine Chemicals, Uppsala, Sweden) (13). The adsorbed IgM preparation (350 µg/ml) agglutinated E at dilutions of up to 1:160. E in Alsever's solution (Colorado Serum Co., Denver, CO) were washed three times in phosphate-buffered saline (PBS), pH 7.3, and were suspended in KRPD. When E were opsonized by incubation with increasing amounts of IgG, release of O_2 (see below) by resident or LPS-elicited macrophages varied directly with the amount of Ig used, over a range of 6.6 µg/ml to 3.3 mg/ml ($n = 5$). A wide range of antibody concentrations was used to sensitize E, as described in Results; concentrations of IgM of 200 or 400 µg/ml agglutinated the E in the tube. Unless otherwise stated, in the experiments reported here, 10^8 E in 1 ml KRPD were incubated with 330 µg of rabbit anti-E IgG or 8.8 µg of rabbit anti-E IgM. After incubation for 15 min at 37°C, the suspensions were centrifuged and washed in and resuspended with KRPD. These preparations are designated E(IgG) or E(IgM). Freshly thawed C5-deficient DBA/2J mouse serum, used as a source of complement, was diluted 1:10 with veronal-buffered saline containing 1 mM Mg^{++}, 0.15 mM Ca^{++}, and 0.1% gelatin (14) and mixed with an equal volume of suspension of E(IgM), 1×10^9/ml, in this buffer. After incubation for 10 min at 37°C, cold KRPD was added to the reaction mixture and the suspension was centrifuged for 5 min at 750 g. The pelleted E were resuspended in KRPD. This preparation is designated E(IgM)C. With the IgM preparation used for most of the experiments reported here, optimal or near optimal ingestion was achieved with a final concentration of mouse serum of 5%.

Glutaraldehyde-treated E(GE) were prepared as described by Capo et al. (15). Three ml of 25% E (vol/vol) in PBS were incubated with 1% glutaraldehyde (Sigma Chemical Co., St. Louis, MO) for 12 h on a rotating wheel at room temperature (15). The suspension was centrifuged and washed six times with PBS and resuspended in KRPD.

Phagocytosis. Ingestion of E(IgG) or E(IgM)C by macrophages was studied as described by Bianco et al. (3) and Griffin et al. (4). The assay was started by the addition of suspensions of 1×10^7 red blood cells (RBC) in 1 ml KRPD to macrophages cultivated overnight on coverslips; the ratio of RBC to macrophages was ~25:1. After incubation for 60 min at 37°C, coverslips were washed with cold KRPD twice, dipped into water for 5 sec to lyse uningested RBC, fixed with 1.25% glutaraldehyde, and examined with phase-contrast microscopy. Each variable was studied with duplicate slides, and at least 100 macrophages were observed with each slide. Results were expressed as the mean number of RBC ingested per macrophage.

Ingestion of GE was studied by a modification of the method of Loike and Silverstein (16). A suspension of 1×10^7 GE in 1 ml KRPD was added to cultured macrophages. After incubation for 60 min at 37°C, the coverslips were washed twice with cold KRPD and inverted over one drop of 0.8 mg/ml trypan blue on a microscope slide. The slides

were immediately observed with a fluorescence microscope with the excitation wavelength set at 490 nm and the emission spectrum set above 515 nm. The discrimination between extracellular GE (which cannot be easily lysed) was based on the observation that extracellular GE fluoresced with a relatively weak red color, whereas intracellular particles maintained a chartreuse color like that of fluorescein (16). The number of ingested RBC was determined for at least 100 macrophages using an oil immersion lens. All preparations were also examined by bright-field and phase-contrast microscopy, and viability was determined by exclusion of trypan blue.

Release of Superoxide Anion. The release of O_2^- was quantitated as the superoxide dismutase-inhibitable reduction of ferricytochrome c (11). The particle-to-macrophage ratio was ~25:1. Each reaction was run in triplicate. Controls contained the same reaction mixture without macrophages. The extent of O_2^- release was expressed in relationship to the mean protein content of three culture plates incubated without E, using the method of Lowry et al. (17) with albumin as standard (11). Macrophage viability was >90% after incubation with E(IgM)C or GE for 90 min at 37°C and >80% after incubation with E(IgG).

Oxygen Consumption. Oxygen consumption during phagocytosis by cells in suspension was measured at 37°C by a polarographic technique using a Clark membrane electrode (12). LPS-elicited peritoneal cells were washed with PBS twice after contaminating E were eliminated by hypotonic lysis (12). Granulocyte-rich preparations were obtained by harvesting ~36 h after intraperitoneal injection of LPS; macrophage-rich preparations were harvested 4 d after injection of LPS (12). The 3 ml reaction mixture contained 1×10^7 peritoneal cells, 2×10^8 RBC, and 2 mM KCN in KRPD. The polarographic tracings were recorded for 10 min after the additon of RBC, and initial rates were measured. Differential counts of exudate cells were performed using Wright's and Giemsa stains.

Results

A comparison of the extent of phagocytosis of E(IgG), E(IgM)C, or GE, and the amount of O_2^- release stimulated by ingestion of these particles, is summarized in Table I. Resident, LPS-elicited, and BCG-activated peritoneal macrophages all ingested large numbers of E(IgG) and released O_2^- actively during phagocytosis.

Release of O_2^- during phagocytosis of E(IgG) was greater in LPS-elicited and BCG-activated macrophages than in resident macrophages (Table I), as shown previously with PMA, candida, or opsonized zymosan as stimulus (11, 12). This relationship was explored further through the comparison of resident and LPS-elicited macrophages. The latter released a greater amount of O_2^- than did resident macrophages at each of eight concentrations of IgG used to make E(IgG) (range, 33 $\mu g/ml$ to 3.3 mg/ml;$n = 2$), at each of the incubation times of 30, 60, 90, and 120 min ($n = 2$), and with ratios of E(IgG)/macrophages of 10:1–100:1 ($n = 3$).

There was only slight phagocytosis of E(IgM)C by resident macrophages, but LPS-elicited and BCG-activated macrophages ingested E(IgM)C effectively (Table I). In contrast, none of the macrophage types released O_2^- during phagocytosis of E(IgM)C (Table I). The same finding was observed when the ratio of E(IgM)C to macrophages was increased to levels of 60:1–150:1 ($n = 3$ with each cell type).

It has been reported (15, 16, 18) that macrophages bind and ingest aldehyde-fixed E in the absence of opsonins, perhaps because cross-linking of red cell membrane components increases local rigidity and modifies hydrophobicity of the red cell surface (15). We studied phagocytosis and O_2^- release stimulated by

TABLE I

Comparison of O_2^- Release and Phagocytosis by Murine Peritoneal
Macrophages*

Type of macrophage	Stimulus	O_2^- released (nmol/mg protein)	Phagocytosis (No. RBC/macrophage)
Resident	E	3 ± 1 (10)	0.01 ± 0 (8)
	E(IgG)	43 ± 5 (9)	8.80 ± 0.87 (6)
	E(IgM)C	4 ± 2 (6)	0.64 ± 0.17 (8)
	GE	3 ± 3 (4)	1.48 ± 0.11 (5)
LPS-elicited	None	4 ± 2 (5)	—
	E	3 ± 1 (15)	0.13 ± 0.03 (5)
	E(IgG)	88 ± 7 (15)	17.40 ± 0.81 (6)
	E(IgM)	2 ± 1 (6)	0.10 ± 0.02 (7)
	E(IgM)C	3 ± 1 (12)	2.33 ± 0.30 (7)
	GE	2 ± 1 (6)	4.36 ± 0.50 (4)
BCG-activated	E	7 ± 1 (6)	0.03 ± 0.01 (5)
	E(IgG)	103 ± 9 (6)	11.80 ± 0.81 (6)
	E(IgM)C	4 ± 1 (6)	1.42 ± 0.25 (5)
	GE	3 ± 2 (5)	2.19 ± 0.28 (4)

* The extent of ingestion and O_2^- release with each of three macrophage
types is compared using E(IgG), E(IgM)C, or GE as stimulus. Incubation
was for 60 min for ingestion or 90 min for O_2^- release. The RBC/
macrophage ratio was ~25:1. Values represent mean ± SEM (n). When
PMA, 500 ng/ml, was used as a stimulus, resident, LPS-elicited, and
BCG-activated macrophages released 83 ± 11 (14), 261 ± 14 (18), and
421 ± 26 (6) nmol O_2^-/mg/90 min, respectively.

GE as a possible model for macrophage clearance of autologous cells. As summarized in Table I, we found that all macrophage types ingested GE relatively effectively, but that ingestion did not stimulate release of O_2^-.

The dissociation between C3 receptor-mediated O_2^- release and ingestion was emphasized by experiments in which E(IgM)C were prepared with different dilutions of fresh, C5-deficient serum used as complement source (Fig. 1). E(IgM) not further opsonized by incubation with fresh serum stimulated no appreciable ingestion or release of O_2^-, as illustrated with LPS-elicited macrophages in Table I. When E(IgM)C were prepared using final concentrations of fresh serum of 2.5–25%, ingestion of the E(IgM)C increased in proportion to the concentration of serum used. However, release of O_2^- in the presence of the E(IgM)C remained at the level obtained with unopsonized E. The amount of IgM used to make E(IgM)C had no effect on either ingestion or O_2^- release over the range of 1–400 μg/ml, using 5 or 10% C5-deficient serum as complement source, presumably because optimal fixation of the relevant opsonin, cleaved C3, was achieved in this system with the lower concentrations of IgM (n = 2, data not shown).

Because ingestion of E(IgM)C was lower than that of E(IgG) when the same numbers of RBC were added (Table I), a reduced number of E(IgG) was incubated with LPS-elicited macrophages so that ingestion of E(IgG) was equivalent to or less than ingestion of E(IgM)C. As shown in Fig. 2, release of O_2^- stimulated by ingestion of decreased numbers of E(IgG) was still at least fivefold
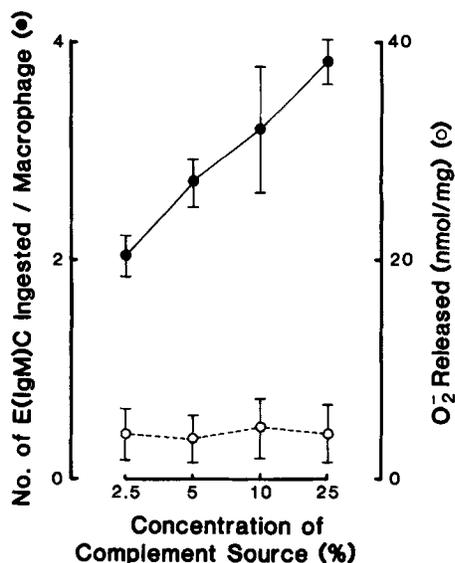
FIGURE 1. Effect of the concentration of the serum used as complement source on the extent of ingestion of E(IgM)C and on E(IgM)C-stimulated O_2^- release by LPS-elicited macrophages. Macrophages were incubated with E(IgM)C, 1×10^7, prepared with different concentrations of serum. The points indicate the means and the bars the SEM of three paired experiments.
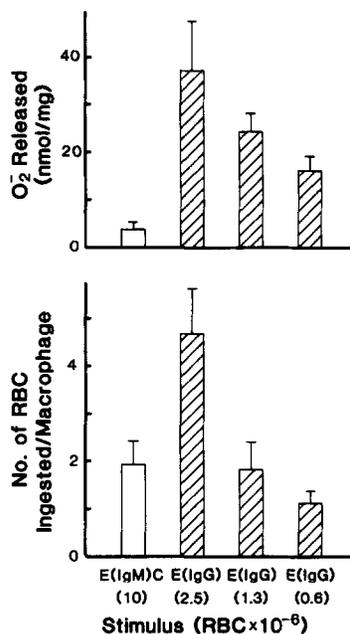

FIGURE 2. Comparison of O_2^- release and ingestion using E(IgM)C and reduced numbers of E(IgG) as stimuli. The number of RBC added to cultured LPS-elicited macrophages is given in parentheses. The columns indicate the means and the bars the SEM of five experiments for O_2^- release or three for ingestion.
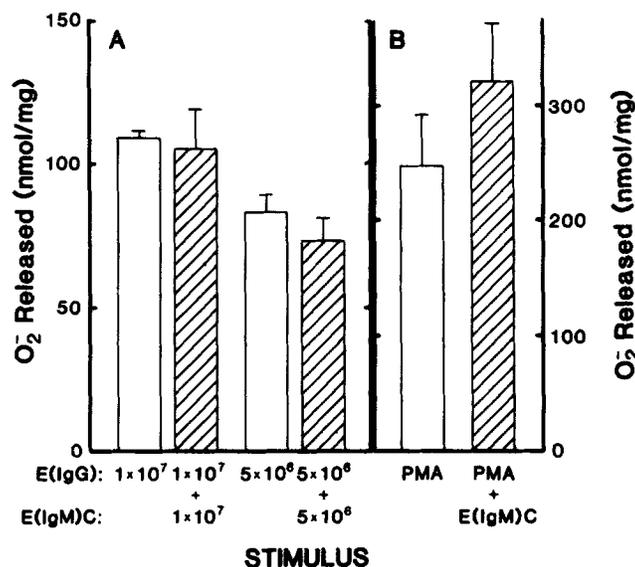
FIGURE 3.   Effect of E(IgM)C on macrophage release of O_2^- stimulated by E(IgG) or PMA. (A) E(IgG) or a mixture of E(IgG) and E(IgM)C were added as indicated. (B) PMA, 500 ng/ml, was used as stimulus in the presence or absence of E(IgM)C. The columns indicate the means and the bars the SEM; the number of experiments was three with 1×10^7 RBC, six with 5×10^6 RBC, and five with PMA.

greater than that stimulated by phagocytosis of E(IgM)C, which was equivalent to the baseline value obtained in the presence of unopsonized E. The relationship between the ingestion of E(IgG) and release of O_2^- was also tested in five experiments with LPS-elicited macrophages by opsonizing E with lower concentrations of IgG (2.2, 3.3, 6.6, 13, 26, and 33 μg/ml). At no concentration tested could ingestion be detected without the concomitant release of O_2^-. For example, with E(IgG) made with 6.6 μg/ml IgG, 1.1 RBC were ingested per macrophage and 29 nmol O_2^- were released per mg cell protein (means of two experiments).

We explored the possibility that the difference in O_2^- release by macrophages exposed to E(IgG) or E(IgM)C might be caused by greater removal of O_2^- by the latter. A mixture of E(IgM)C and E(IgG), or of E(IgM)C and PMA, was added to LPS-elicited macrophages as a stimulus. When exposed to a mixture of E(IgG) and E(IgM)C, either 1×10^7 or 5×10^6 each, macrophages released an equivalent amount of O_2^- to that stimulated by the same number of E(IgG) alone (Fig. 3A). When LPS-elicited macrophages were exposed to PMA and E(IgM)C at the same time, even more O_2^- release was observed than with PMA alone (Fig. 3B). Additional evidence that E(IgM)C or GE did not scavenge O_2^- or inhibit function of the enzyme responsible for the respiratory burst in macrophages was obtained by studying O_2^- release by LPS-elicited macrophages exposed to these E further coated with IgG. In four paired experiments with LPS-elicited macrophages, O_2^- release stimulated by E(IgM)C-IgG (99.2 ± 16.4 nmol/mg) was equivalent to that obtained with E(IgG) (107 ± 12.7 nmol/mg). In contrast to the lack of stimulation of O_2^- release seen with GE (Table I), GE coated with IgG stimulated the release of O_2^- effectively (69.1 ± 8.9 nmol/mg, $n = 5$).

TABLE II

*Comparison of the Capacity of E(IgG), E(IgM)C, and GE to Stimulate Macrophage Oxygen Uptake**

Stimulus	Oxygen consumed	
	Macrophage preparations	Granulocyte preparations
	$nmol/10^7$ cells/min[‡]	
E	0.7 ± 0.2 (4)	0.6 ± 0.1 (3)
E(IgG)	6.8 ± 0.3 (5)	10.5 ± 1.1 (3)
E(IgM)C	1.0 ± 0.2 (6)	4.5 ± 0.5 (3)
GE	1.2 ± 0.4 (5)	1.2 ± 0.1 (4)

* LPS-elicited peritoneal cells, 1×10^7, were tested in suspension, as required for this assay. In the macrophage preparations, 81% of the phagocytes were macrophages and 19% were granulocytes (means, n = 5; range, 74–86%). In the granulocyte preparations, a mean of 70% of the phagocytes were granulocytes (range, 61–82%, n = 3). A mean of 42% of the cells in macrophage preparations and a mean of 31% in neutrophil preparations were lymphocytes. KCN, 2 mM, and 1×10^8 RBC were added to begin the reaction. Oxygen consumption by E or GE was negligible in the presence of 2 mM KCN. When stimulated with PMA, 0.5 µg/ml, macrophage preparations consumed 17.7 ± 0.5 nmol oxygen/10^7 cells/min (n = 4).

‡ Results are expressed as mean ± SEM (n).

Final evidence for the dissociation of phagocytosis of E(IgM)C or GE from stimulation of the respiratory burst was obtained by studying oxygen consumption by LPS-elicited murine peritoneal cells exposed to those stimuli (Table II). There was a consistent and marked increase in oxygen consumption when macrophage-rich preparations were incubated with E(IgG) but minimal consumption in the presence of E(IgM)C or GE. This minimal consumption might be explained by the presence in the macrophage preparations of neutrophils, which appeared to be stimulated by E(IgM)C and GE. The stimulation achieved by E(IgG) with LPS-elicited cell preparations rich in neutrophils was greater than that with the macrophage-rich preparations (Table II), but the results obtained with macrophage preparations exposed to E(IgG) could not be attributed to the activity of contaminating granulocytes. Comparable respiratory responses by murine peritoneal macrophages and granulocytes have been reported previously using candida (11), PMA (19), and zymosan particles (20) as stimuli.

Discussion

Previous studies (8, 9, 21, 22) with neutrophils have indicated that ingestion of particles through Fc or C3b receptors (or both) is invariably associated with the stimulation of oxidative metabolism. Moreover, the extent of the oxidative response has varied directly with the extent of ingestion (8, 21, 22). Phagocytosis of unopsonized candida and certain protozoa, bacteria, and inert particles by neutrophils or mononuclear phagocytes has also been shown to stimulate oxidative metabolism vigorously (12, 23–29), although the involvement of a receptor in the process has not been clearly defined.

Studies in other systems, however, suggest that under certain circumstances phagocytosis is not associated with an equivalent stimulation of the respiratory burst. For example, a dissociation between phagocytosis of complement-coated particles and the oxidative response has been shown to exist in cells of a continuous tissue culture line derived from a patient with acute myeloblastic leukemia (30). A similar dissociation was created pharmacologically by incubating normal neutrophils with a relatively small concentration of cytochalasin B (1 μg/ml), which inhibited the ingestion of serum-treated yeasts by only 5% but decreased the concurrent release of O_2^- by 75% (31). A relative dissociation has been shown between the ingestion of two species of candida and their capacity to stimulate the respiratory burst in peritoneal macrophages (12). Although the ingestion of unopsonized C. albicans and C. parapsilosis was equivalent, C. albicans, which was killed relatively poorly by macrophages, stimulated the respiratory burst much more weakly than did C. parapsilosis. Preincubation of gelatin-coated latex or zymosan in plasma fibronectin and heparin improved phagocytosis of these particles by elicited rat neutrophils, as estimated by light and electron microscopy, but enhanced only slightly the hexose monophosphate shunt and chemiluminescence responses to these particles, suggesting that heparin/fibronectin-mediated ingestion could be dissociated from the respiratory burst (32). Although treatment of phagocytes with partially purified neuraminidase was reported to inhibit the release of O_2^- (33) or H_2O_2 (34) without decreasing phagocytosis, this effect was not achieved with purified neuraminidase (35) or when the enzyme was removed before assay of oxidative metabolism (25).

The results reported here indicate that stimulation of macrophages through Fc receptor-mediated phagocytosis of E(IgG) triggers the expected oxidative response, manifested as vigorous consumption of oxygen and elaboration of O_2^-. In contrast, phagocytosis of approximately equivalent numbers of E(IgM)C or E treated with glutaraldehyde (GE) failed to stimulate the respiratory burst. An inability of C3b or C3bi attached to surfaces to stimulate release of hydrogen peroxide has been found recently with human neutrophils, monocytes, and monocyte-derived macrophages (36).

The biochemical events that link receptor-ligand binding at the membrane of a phagocytic cell and the subsequent processes of endocytosis, degranulation, and oxidative metabolism are not known. The complete dissociation between phagocytosis and the oxidative burst shown here with C3b-coated or glutaraldehyde-treated E suggests that the pathway linking membrane binding to phagocytosis is different from that linking binding to stimulation of the enzyme responsible for the respiratory burst. Binding through the Fc receptor would appear to stimulate both pathways in both neutrophils and macrophages (although phagocytosis is not required to induce the release of oxygen metabolites by phagocytes bound to IgG-coated surfaces (36-38)). In contrast, attachment to macrophages through the receptor for C3b or through nonspecific chemical bonds appears to stimulate the cell's contractile machinery without triggering the respiratory burst. Electron microscopic analysis of the mechanism of internalization of E(IgG) and E(IgM)C by rat Kupffer cells and mouse peritoneal macrophages suggests clearcut differences that may relate to our findings, including a paucity of cytoplasmic extensions and much less contact between the

macrophage plasma membrane and the erythrocyte during ingestion of E(IgM)C (39, 40). Study of the biochemical mechanisms by which macrophages respond to the ingestion of E opsonized with IgG or C3b could provide a basis for further elucidation of the molecular events that mediate the microbicidal activity of phagocytic cells.

Macrophages elicited by LPS or activated by BCG infection released over twice as much O_2^- as did resident cells on exposure to E(IgG) (Table I). Thus, stimulation of oxidative metabolism through the Fc receptor, like stimulation by PMA, zymosan, bacteria, candida, or toxoplasma (12, 28, reviewed in 41), is greater with elicited or activated macrophages than with resident macrophages.

The generation of highly reactive oxygen intermediates during phagocytosis is believed to be essential for phagocytic microbicidal activity. However, toxic oxygen metabolities are released to the outside during this process (11, 12, 19, 20, 29). Therefore, the potential exists for oxidative damage to surrounding tissues (37, 38), and such has been demonstrated to occur (reviewed in 42). Moreover, the oxidative metabolic response has been shown to markedly reduce the chemotactic, phagocytic, and metabolic capacities of the phagocytes themselves (43, 44). The data reported here illustrate clearly that phagocytosis by macrophages is not always accompanied by a respiratory burst. Whether removal of some or all effete cells by macrophages in vivo requires opsonization by autoantibody is not clear (45–47). Thus, it is possible that phagocytic removal of particles not "foreign" enough to elicit an IgG antibody response, including damaged or aged cells, might occur in tissues or in the mononuclear phagocyte system without inflicting unnecessary oxidative damage to surrounding cells or to the phagocyte itself.

Summary

We explored the relationship between phagocytosis and the triggering of oxidative metabolism using resident, lipopolysaccharide (LPS)-elicited, and ba-cille Calmette-Guérin (BCG)-activated murine peritoneal macrophages. Sheep erythrocytes (E) coated with IgG [E(IgG)], E coated with IgM and complement [E(IgM)C], and E treated with 1% glutaraldehyde (GE) were used as stimuli. All three types of macrophages released superoxide anion (O_2^-) during phagocytosis of E(IgG). All macrophage types phagocytosed E(IgM)C and GE but none were stimulated to release O_2^- during phagocytosis of these particles. Vigorous consumption of oxygen was also stimulated by the ingestion of E(IgG) but not by ingestion of E(IgM)C or GE. E(IgM)C did not scavenge the O_2^- released from macrophages during phagocytosis of E(IgG) or during exposure to phorbol myristate acetate, and further addition of IgG anti-E antibody to E(IgM)C or GE permitted optimal stimulation of macrophage O_2^- release by these particles. The capacity of macrophages to ingest E(IgM)C and GE without stimulating the respiratory burst raises the possibility that clearance of particulate matter not opsonized with specific IgG might be achieved without stimulation of the release of toxic oxygen metabolites, and, therefore, without the risk of oxidative damage to the phagocytic cell or surrounding tissue.

References

1. Mantovani, B., M. Rabinovitch, and V. Nussenzweig. 1972. Phagocytosis of immune complexes by macrophages: different roles of the macrophage receptor sites for complement (C3) and for immunoglobulin (IgG). *J. Exp. Med.* 135:780.

2. Rabinovitch, M. 1970. Phagocytic recognition. *In* Mononuclear Phagocytes. R. van Furth, editor. Blackwell Scientific Publications, Oxford. 299–315.

3. Bianco, C., F. M. Griffin, Jr., and S. C. Silverstein. 1975. Studies of the macrophage complement receptor: alteration of receptor function upon macrophage activation. *J. Exp. Med.* 141:1278.

4. Griffin, F. M., Jr., C. Bianco, and S. C. Silverstein. 1975. Characterization of the macrophage receptor for complement and demonstration of its functional independence from the receptor for the Fc portion of immunoglobulin G. *J. Exp. Med.* 141:1269.

5. Edelson, P. J., and C. Erbs. 1978. Biochemical and functional characteristics of the plasma membrane of macrophages from BCG-infected mice. *J. Immunol.* 120:1532.

6. Morland, B., and G. Kaplan. 1977. Macrophage activation in vivo and in vitro. *Exp. Cell Res.* 108:279.

7. Mantovani, B. 1981. Phagocytosis of immune complexes mediated by IgM and C3 receptors by macrophages from mice treated with glycogen. *J. Immunol.* 126:127.

8. Newman, S. L., and R. B. Johnston, Jr. 1979. Role of binding through C3b and IgG in polymorphonuclear neutrophil function: studies with trypin-generated C3b. *J. Immunol.* 123:1839.

9. Roos, D., A. A. M. Bot, M. L. J. van Schaik, M. de Boer, and M. R. Daha. 1981. Interaction between human neutrophils and zymosan particles: the role of opsonins and divalent cations. *J. Immunol.* 126:433.

10. Danley, D. L., and A. E. Hilger. 1981. Stimulation of oxidative metabolism in murine polymorphonuclear leukocytes by unopsonized fungal cells: evidence for a mannose-specific mechanism. *J. Immunol.* 127:551.

11. Johnston, R. B., Jr., C. A. Godzik, and Z. A. Cohn. 1978. Increased superoxide anion production by immunologically activated and chemically elicited macrophages. *J. Exp. Med.* 148:115.

12. Sasada, M., and R. B. Johnston, Jr. 1980. Macrophage microbicidal activity: correlation between phagocytosis-associated oxidative metabolism and the killing of candida by macrophages. *J. Exp. Med.* 152:85.

13. Bianco, C., and B. Pytowski. 1981. Fc and C3 receptors. *In* Methods for Studying Mononuclear Phagocytes. D. O. Adams, P. J. Edelson, and H. S. Koren, editors. Academic Press, Inc., New York. 273–282.

14. Rapp, H. J., and T. Borsos. 1970. Molecular Basis of Complement Action. Appleton-Century-Crofts, New York. 75.

15. Capo, C., P. Bongrand, A.-M. Benoliel, and R. Depieds. 1979. Non-specific recognition in phagocytosis: ingestion of aldehyde-treated erythrocytes by rat peritoneal macrophages. *Immunology.* 36:501.

16. Loike, J. D., and S. C. Silverstein. 1983. A fluorescence quenching technique using trypan blue to differentiate between attached and ingested glutaraldehyde-fixed red blood cells in phagocytosing murine macrophages. *J. Immunol. Methods.* 57:373.

17. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193:265.

18. Rabinovitch, M. 1967. Attachment of modified erythrocytes to phagocytic cells in absence of serum. *Proc. Soc. Exp. Biol. Med.* 124:396.

19. Nathan, C. F., and R. K. Root. 1977. Hydrogen peroxide release from mouse peritoneal macrophages: dependence on sequential activation and triggering. *J. Exp. Med.* 146:1648.

20 Drath, D. B., and M. L. Karnovsky. 1975. Superoxide production by phagocytic leukocytes. *J. Exp. Med.* 141:257.

21. Johnston, R. B., Jr., M. R. Klemperer, C. A. Alper, and F. S. Rosen. 1969. The enhancement of bacterial phagocytosis by serum: the role of complement components and two cofactors. *J. Exp. Med.* 129:1275.

22. Stossel, T. P. 1973. Evaluation of opsonic and leukocyte function with a spectrophotometric test in patients with infection and with phagocytic disorders. *Blood.* 42:121.

23. Diamond, R. D., and R. Krzesicki. 1978. Mechanisms of attachment of neutrophils to *Candida albicans* pseudohyphae in the absence of serum, and of subsequent damage to pseudohyphae by microbicidal processes of neutrophils in vitro. *J. Clin. Invest.* 61:360.

24. Diamond, R. D., and Haudenschild, C. C. 1981. Monocyte-medicated serum-independent damage to hyphal and pseudohyphal forms of *Candida albicans* in vitro. *J. Clin. Invest.* 67:173.

25. Johnston, R. B., Jr., D. A. Chadwick, and Z. A. Cohn. 1981. Priming of macrophages for enhanced oxidative metabolism by exposure to proteolytic enzymes. *J. Exp. Med.* 153:1678.

26. Stubbs, M., A. V. Kühner, E. A. Glass, J. R. David, and M. L. Karnovsky. 1973. Metabolic and functional studies on activated mouse macrophages. *J. Exp. Med.* 137:537.

27. Wilson, C. B., V. Tsai, and J. S. Remington. 1980. Failure to trigger the oxidative metabolic burst by normal macrophages: possible mechanism for survival of intracellular pathogens. *J. Exp. Med.* 151:328.

28. Murray, H. W., and Z. A. Cohn. 1980. Macrophage oxygen-dependent antimicrobial activity. III. Enhanced oxidative metabolism as an expression of macrophage activation. *J. Exp. Med.* 152:1596.

29. Murray, H. W. 1981. Interaction of *Leishmania* with a macrophage cell line: correlation between intracellular killing and the generation of oxygen intermediates. *J. Exp. Med.* 153:1690.

30. Newburger, P. E., J. S. Pagano, J. S. Greenberger, A. Karpas, and H. J. Cohen. 1980. Dissociation of opsonized particle phagocytosis and respiratory burst activity in an Epstein-Barr virus-infected myeloid cell line. *J. Cell Biol.* 85:549.

31. Hed, J., and O. Stendahl. 1982. Differences in the ingestion mechanisms of IgG and C3b particles in phagocytosis by neutrophils. *Immunology.* 45:727.

32. Gudewicz, P. W., D. H. Beezhold, P. Van Alten, and J. Molnar. 1982. Lack of stimulation of post-phagocytic metabolic activities of polymorphonuclear leukocytes by fibronectin opsonized particles. *J. Reticuloendothel. Soc.* 32:143.

33. Tsan, M.-F., and P. A. McIntyre. 1976. The requirement for membrane sialic acid in the stimulation of superoxide production during phagocytosis by human polymorphonuclear leukocytes. *J. Exp. Med.* 143:1308.

34. Tsan, M.-F., K. H. Douglass, and P. A. McIntyre. 1977. Hydrogen peroxide production and killing of *Staphylococcus aureus* by human polymorphonuclear leukocytes. *Blood.* 49:437.

35. Suzuki, H., T. Kurita, and K. Kakinuma. 1982. Effects of neuraminidase on O_2^- consumption and release of O_2^- and H_2O_2 from phagocytosing human polymorphonuclear leukocytes. *Blood.* 60:446.

36. Wright, S. D., and S. C. Silverstein. Receptors for C3b and C3bi promote phagocytosis but not the release of toxic oxygen from human phagocytes. *J. Exp. Med.* 158:2016.

37. Johnston, R. B., Jr., J. E. Lehmeyer, and L. A. Guthrie. 1976. Generation of superoxide anion and chemiluminescence by human monocytes during phagocytosis and on contact with surface-bound immunoglobulin G. *J. Exp. Med.* 143:1551.

38. Johnston, R. B., Jr., and J. E. Lehmeyer. 1976. Elaboration of toxic oxygen byproducts by neutrophils in a model of immune complex disease. *J. Clin. Invest.* 57:836.

39. Munthe-Kaas, A. C., G. Kaplan, and R. Seljelid. 1976. On the mechanism of internalization of opsonized particles by rat Kupffer cells in vitro. *Exp. Cell Res.* 103:201.

40. Kaplan, G. 1977. Differences in the mode of phagocytosis with Fc and C3 receptors in macrophages. *Scand. J. Immunol.* 6:797.

41. Johnston, R. B., Jr. 1981. Enhancement of phagocytosis-associated oxidative metabolism as a manifestation of macrophage activation. *Lymphokines.* 3:33.

42. Fantone, J. C., and P. A. Ward. 1982. Role of oxygen-derived free radicals and metabolites in leukocyte-dependent inflammatory reactions. *Am. J. Pathol.* 107:397.

43. Baehner, R. L., L. A. Boxer, J. M. Allen, and J. Davis. 1977. Autooxidation as a basis for altered function by polymorphonuclear leukocytes. *Blood.* 50:327.

44. Roos, D., R. S. Weening, A. A. Voetman, M. L. J. van Schaik, A. A. M. Bot, L. J. Meerhof, and J. A. Loos. 1979. Protection of phagocytic leukocytes by endogenous glutathione: studies in a family with glutathione reductase deficiency. *Blood.* 53:851.

45. Kay, M. M. B. 1975. Mechanism of removal of senescent cells by human macrophages in situ. *Proc. Natl. Acad. Sci. USA* 72:3521.

46. Newman, S. L., J. E. Henson, and P. M. Henson. 1982. Phagocytosis of senescent neutrophils by human monocyte-derived macrophages and rabbit inflammatory macrophages. *J. Exp. Med.* 156:430.

47. Lutz, H. U., and G. Wipf. 1982. Naturally occurring autoantibodies to skeletal proteins from human red blood cells. *J. Immunol.* 128:1695.