

STUDIES OF THE MACROPHAGE COMPLEMENT RECEPTOR

Alteration of Receptor Function upon Macrophage Activation*

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Macrophages possess on their plasma membranes receptors for the Fc portion of IgG and for the third complement of complement (1,2). These receptors are important in the performance of some of the functions of these cells. The Fc receptors mediate both attachment and ingestion of IgG-coated particles (3,4). However, the complement receptors of mouse peritoneal macrophages and of human and rabbit alveolar macrophages mediate binding of C3-coated particles to the macrophage surface but do not appear to mediate ingestion of these particles (3,5,6).

In several experimental systems, immunization or chronic intracellular infection has been shown to alter certain functions of the immunized or infected animal's macrophages (7-13). Intraperitoneal injection of some foreign substances such as thioglycollate medium and bacillus Calmette-Guérin (BCG)¹ effects functional changes in the animal's peritoneal macrophages similar to those changes induced by immunization or intracellular infection. Macrophages from such animals are termed activated macrophages. They spread more readily on glass; contain more mitochondria, lysosomes, and lysosomal enzymes; demonstrate increased spontaneous and postphagocytic glucose carbon-1 oxidation; and possess greater phagocytic, microbicidal, and microbistatic activity than their nonactivated counterparts (7-13). A qualitative difference in enzyme function between activated and nonactivated macrophages was recently demonstrated by Unkeless et al. (14). They found that macrophages from mice injected intraperitoneally with thioglycollate medium produce and secrete a neutral protease, plasminogen activator, while macrophages from control mice do not.

In this paper, we present the results of our studies of the functions of the Fc receptors and complement receptors of activated and nonactivated mouse peritoneal macrophages. We find that the quantity of ingestion mediated by the Fc

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¹ *Abbreviations used in this paper:* BCG, bacillus Calmette-Guérin; E, sheep erythrocytes; E(IgG), E coated with anti-E IgG; E(IgG)C, E(IgG) coated with the first four complement components; E(IgM), E coated with anti-E IgM; E(IgM)C, E(IgM) coated with the first four complement components; PBS, phosphate-buffered saline with Ca⁺⁺ and Mg⁺⁺ ions; PD, PBS without Ca⁺⁺ and Mg⁺⁺ ions; SAR IgG, IgG fraction of serum from a sheep immunized with rabbit F(ab')₂ immunoglobulin fragments; VBG, Veronal-buffered glucose with Ca⁺⁺, Mg⁺⁺, and 0.1% gelatin.

receptors is greater in activated than in nonactivated macrophages. The complement receptors of nonactivated macrophages mediate only particle binding to the macrophage surface while the complement receptors of activated cells mediate both attachment and ingestion of complement-coated particles.

Materials and Methods

Animals. Mouse erythrocytes and peritoneal macrophages were obtained from Swiss mice maintained at The Rockefeller University, N.Y. C5-deficient mouse serum was prepared from a C5-deficient strain of AKR mice (Jackson Laboratories, Bar Harbor, Maine).

Erythrocytes. Sheep erythrocytes (E) in Alsever's solution (Animal Blood Center, Syracuse, N.Y.) were washed three times in PD [solution "a" of Dulbecco's phosphate-buffered saline (PBS) (15)] and suspended in medium 199 (Microbiological Associates, Bethesda, Md.) or in Veronal-buffered glucose containing Ca^{++} , Mg^{++} , and 0.1% gelatin (VBG) (16).

Macrophage activator. Brewer thioglycollate medium was obtained from Difco Laboratories, Detroit, Mich., and a 4.05% aqueous solution prepared according to the manufacturer's instructions.

Macrophages. The methods for harvesting and maintaining mouse peritoneal macrophages were those of Cohn and Benson (17), as previously modified (18). Cells were cultivated for 24–48 h in medium 199 with 20% heat-decomplemented (56°C, 30 min) fetal bovine serum (Grand Island Biological Co., Grand Island, N.Y.), 100 U/ml of penicillin, and 100 $\mu\text{g}/\text{ml}$ of streptomycin. In some experiments, mice were injected intraperitoneally with 1 ml of Brewer thioglycollate medium 4 days before their peritoneal macrophages were harvested. Macrophages from these animals are designated activated. Latex beads, 1.1 μ in diameter (Dow Chemical Co., Midland, Mich.) were washed three times in PD, suspended in PD at a concentration of 6×10^8 particles/ml, and stored at 4°C.

Sera. 5 ml of normal rabbit serum was adsorbed at 4°C for 2 h with 10^{10} mouse erythrocytes. The adsorbed serum was frozen at –70°C and used as a source of complement for cytotoxic assays.

Serum was prepared from C5-deficient AKR mice and stored immediately in 0.2 ml aliquots at –70°C. It served as a source of C1423 in all experiments involving the use of complement-coated E.

Antisera and Immunoglobulin Fractions

RABBIT ANTIMOUSE MACROPHAGE IgG. Rabbit antimouse macrophage serum, prepared as previously described (18,19), was chromatographed on DEAE cellulose. The resulting IgG fraction was dialyzed against PBS, concentrated by vacuum dialysis, Millipore filtered (0.45 μ pore size), and stored at 4°C. The protein concentration of this IgG fraction was 12 mg/ml; it agglutinated mouse erythrocytes at a dilution of 1:500.

RABBIT ANTIMOUSE MACROPHAGE F(ab')₂. The F(ab')₂ fragment of rabbit antimouse macrophage IgG was prepared by pepsin digestion, using the method of Nisonoff (20), as previously described (18). The protein concentration of this F(ab')₂ fraction was 3 mg/ml; it agglutinated mouse erythrocytes at a dilution of 1:32.

Sheep IgG prepared against rabbit F(ab')₂ (SAR IgG) was prepared and purified as previously described (18). The protein concentration of SAR IgG was 28 mg/ml.

Rabbit anti-sheep E IgG (lot no. 10154), at a concentration of 5 mg/ml was obtained from Cordis Laboratories, Miami, Fla. It agglutinated sheep E at a dilution of 1:3,000.

Rabbit anti-sheep E IgM (lot no. 30704), at a concentration of 350 $\mu\text{g}/\text{ml}$, was also obtained from Cordis Laboratories. It agglutinated sheep E at a dilution of 1:320.

Preparation of Immune Complexes. 1 ml of 5% (vol/vol) E in medium 199 was incubated with either 125 μg of rabbit anti-sheep E IgG or 13 μg of rabbit anti-sheep E IgM for 15 min at 37°C. The suspensions were centrifuged for 5 min at 750 *g* and the pellets suspended to 0.5% in medium 199. These preparations (0.5% E coated with anti-E IgG or with anti-E IgM) are designated E(IgG) and E(IgM), respectively.

Preparation of Complement-Coated Immune Complexes. Portions of 5% E(IgG) and E(IgM) were suspended in VBG and mixed with an equal volume of a 1:10 dilution of freshly thawed C5-deficient AKR mouse serum in VBG. The mixtures were incubated for 10 min at 37°C and washed twice by centrifugation for 5 min at 750 *g*. The pelleted erythrocytes were suspended to 0.5% in medium 199. These preparations [0.5% complement-coated E(IgG) and 0.5% complement-coated

E(IgM)] are designated E(IgG)C and E(IgM)C, respectively. VBG and all erythrocyte preparations were made fresh on the day of an experiment.

Trypsin Treatment of Macrophages. Trypsin from bovine pancreas, Type III, no. T-8253, was obtained from Sigma Chemical Co., St. Louis, Mo. Macrophage monolayers were incubated for 30 min at 37°C with 1 mg/ml of trypsin in medium 199. Cover slips were washed twice with 1 mg/ml of ovomucoid trypsin inhibitor (Worthington Biochemical Corp., Freehold, N. J.) in medium 199 to terminate trypsin digestion.

Phase-Contrast and Electron Microscopy. Cover slip cultures were fixed with glutaraldehyde and examined by phase-contrast and electron microscopy as previously described (18). Cells were processed for electron microscopy as described (21, 22) and examined in a Siemens Elmiskop 1A (Siemens Corp., Medical Industrial Groups, Iselin, N.J.).

Assay System. Macrophage monolayers were cultivated for 24–48 h on 13-mm diameter glass cover slips. Cover slips were washed with medium² and covered with either medium or 0.05 ml of antimacrophage IgG (6 µg protein) or antimacrophage F(ab')₂ (6 µg protein) for 2 h at 37°C. After washing, 2 ml of medium and 0.2 ml of one of the various erythrocyte preparations or latex particles were added to each 35 mm petri dish, and incubation was continued for 30 min at 37°C. Preparations were fixed and examined. At least three separate experiments, performed in duplicate, are represented by each value presented. At least 100 macrophages were evaluated for each determination.

In studies examining complement-mediated macrophage lysis, monolayers were incubated with either medium alone, antimacrophage IgG, or antimacrophage F(ab')₂, as above. Some macrophage preparations were incubated with SAR IgG or with antimacrophage F(ab')₂ and then with SAR IgG. The monolayers were then washed and incubated with 5% (vol/vol) freshly thawed rabbit serum (a source of hemolytic complement) in medium for 30 min at 37°C and the percentage of macrophages lysed determined by phase-contrast microscopy and by trypan blue exclusion.

Presentation of Results. The data from experiments examining the interaction of macrophages with the various erythrocyte preparations are presented as follows: percent ingestion or attachment is the percentage of macrophages with erythrocytes ingested or attached. Ingestion or attachment index is the percentage of macrophages which ingested or attached erythrocytes × average number of erythrocytes ingested or attached per macrophage.

Miscellaneous. Hemagglutination titers of IgG, IgM, and F(ab')₂ preparations were determined as previously described (18). Protein determinations were performed by the method of Lowry et al. (23), using bovine serum albumin as a standard. VBG was prepared as described by Rapp and Borsos (16).

Results

Attachment and Ingestion of Sensitized Sheep Erythrocytes by Nonactivated Macrophages. The attachment and ingestion indices obtained when various preparations of sheep erythrocytes were incubated with 24 h explanted nonactivated mouse peritoneal macrophages are shown in Fig. 1. Neither E nor E(IgM) were bound or ingested by these macrophages; E(IgM)C were attached but were not ingested. The usual period of incubation of the various erythrocyte preparations with macrophages was 30 min; prolonging the incubation period to 3 h did not produce an appreciable change in the number of erythrocytes attached or ingested. Similar experiments were performed with macrophages cultivated for 2, 48, and 72 h, and, in all cases, E(IgM)C attached to the macrophage plasma membrane but were not ingested in significant numbers. To confirm that E(IgM)C were not ingested, we incubated some preparations with 0.83% ammonium chloride just before fixation. Incubation with ammonium chloride causes lysis of extracellular erythrocytes, has no effect upon the macrophages or their content of phagocytized erythrocytes, and allows the intracellular erythro-

²Throughout this paper the term "medium" refers to medium 199.

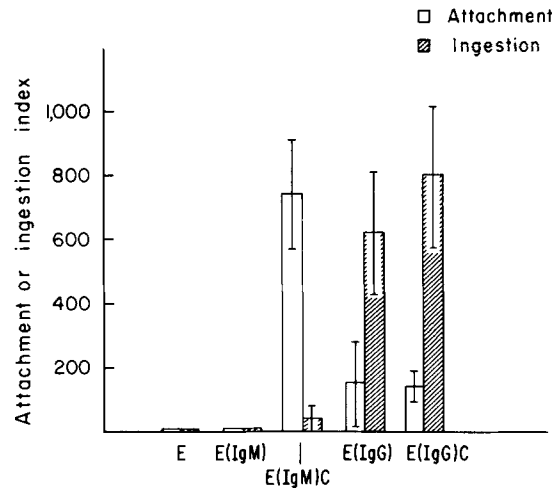


FIG. 1. Attachment and ingestion of preparations of sensitized sheep erythrocytes by nonactivated macrophages. The bars for attachment represent only those macrophages which have attached but not ingested erythrocytes. Lines represent the standard errors of the means.

cytes to be counted without interference by extracellular erythrocytes. Virtually all erythrocytes in these preparations were lysed by ammonium chloride treatment, indicating that E(IgM)C were not ingested by macrophages. Additional confirmation was obtained by examining parallel cultures by electron microscopy. E(IgG) were avidly ingested; the addition of mouse complement to these preparations [E(IgG)C] did not significantly change the fate of these sensitized erythrocytes.

It should be noted that the presence of small amounts of IgG contaminating the IgM fraction led to ingestion of sensitized erythrocytes. For example, E(IgM) and E(IgM)C prepared with one IgM fraction (lot no. 40324 from Cordis Laboratories) were ingested by nonactivated (and by activated) macrophages. To determine the presence of IgG-contaminating IgM fractions, we coated E with a subagglutinating titer of the IgM fraction and determined the ability of an antibody prepared against the Fc fragment of rabbit IgG to agglutinate the coated erythrocytes. The agglutination titer of anti-Fc IgG for E(IgM) prepared with IgM lot no. 40324 was 1:128. When this anti-Fc antibody failed to agglutinate E(IgM), as was the case when IgM fraction (lot no. 30704 from Cordis Laboratories) was used, minimal ingestion of E(IgM) or E(IgM)C by nonactivated macrophages was observed, as indicated in Fig. 1.

Ingestion of E(IgG) and E(IgG)C by Activated Macrophages. Activated macrophages were more efficient in phagocytizing IgG-coated erythrocytes than their nonactivated counterparts. As shown in Fig. 2, 24-h and 48-h explanted activated macrophages ingested both E(IgG) and E(IgG)C more avidly than did nonactivated cells. The figure also shows that cultivation of nonactivated macrophages under the described conditions also leads to more active ingestion of IgG-coated erythrocytes, though the difference between activated and nonactivated macrophages remains evident. During cultivation, morphological changes also occur, principally better cell spreading, increased membrane ruffling, and increased numbers of phase-dense cytoplasmic granules.

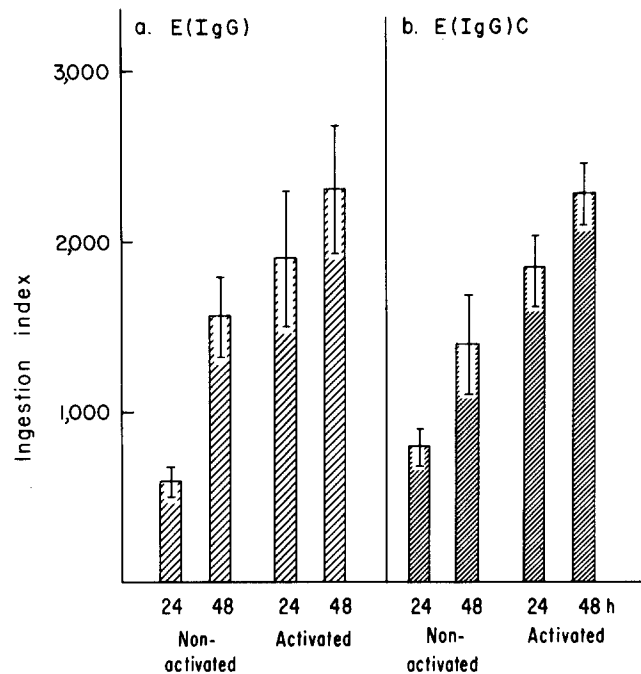


FIG. 2. Ingestion by activated and by nonactivated macrophages of IgG-coated sheep erythrocytes prepared with and without complement. Lines represent the standard errors of the means.

Modification of the Functional Activity of Complement Receptors upon Macrophage Activation. While the phagocytosis of E(IgG) by macrophages is quantitatively enhanced by thioglycollate medium activation, the functional activity of complement receptors of activated macrophages is qualitatively different from that of nonactivated macrophages. As shown in Fig. 1, nonactivated macrophages do not ingest appreciable numbers of E(IgM)C. Activated macrophages, however, ingest large numbers of E(IgM)C. Fig. 3 illustrates the ingestion indices obtained when E(IgM) or E(IgM)C were incubated with activated and with nonactivated macrophages which had been cultivated for 24 or 48 h. 66% of 24-h explanted activated macrophages ingested an average of 7.8 E(IgM)C each (ingestion index of 518), while 89% of 48-h explanted activated macrophages ingested an average of 11.1 E(IgM)C each (ingestion index of 986). 24-h and 48-h explanted nonactivated macrophages gave ingestion indices of 38 and 62, respectively. The difference between the results obtained with activated and with nonactivated macrophages is highly significant³ with no overlapping values. Similar results were obtained with 2-h and 72-h explanted activated and nonactivated macrophages. Binding and ingestion of E and E(IgM) by activated macrophages was low (ingestion index lower than 80), but was greater than the binding observed with nonactivated macrophages.

Blocking of Fc Receptors by an Antimacrophage IgG Fraction. In order to eliminate the participation of erythrocyte-bound IgG and receptors for the Fc

³ $P < 0.001$ by the Student's t test for both 24 and 48 h explanted macrophages.

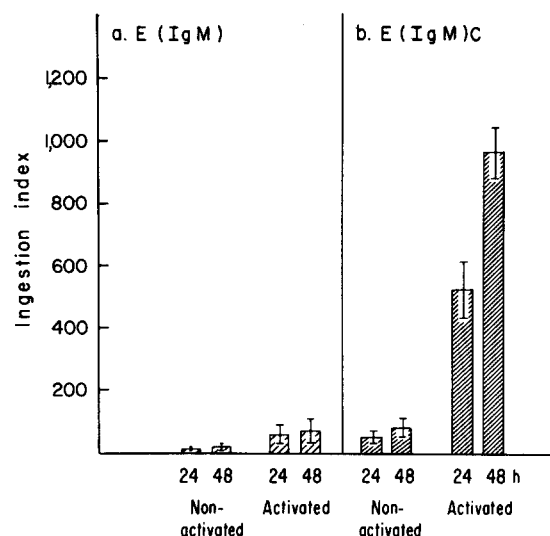


FIG. 3. Ingestion by activated and by nonactivated macrophages of IgM-coated sheep erythrocytes prepared with and without complement. Lines represent the standard errors of the means.

portion of IgG in the interaction of macrophages with complement-coated erythrocytes, we blocked the Fc receptors of the macrophage plasma membrane with antimacrophage IgG. As shown by Holland et al. (19), this antimacrophage IgG blocks ingestion mediated by the Fc receptor, but has no effect upon ingestion of formaldehyde-treated erythrocytes.

We prepared antimacrophage IgG and its $F(ab')_2$ fragment, characterized the binding of these immunoglobulin fractions to the macrophage surface, and examined their effects upon the phagocytic capacities of the macrophage. Table I summarizes the cytotoxic abilities of antimacrophage IgG and antimacrophage $F(ab')_2$. Both antimacrophage IgG and antimacrophage $F(ab')_2$ bound to the macrophage plasma membrane as measured by their abilities to directly or indirectly [as is the case with antimacrophage $F(ab')_2$], mediate macrophage lysis in the presence of hemolytic complement. The antigenic determinants on the macrophage surface to which these antibodies are directed are not removed by incubation of the macrophages with up to 1 mg/ml of trypsin for 30 min at 37°C.

Table II demonstrates the effects of antimacrophage IgG and of antimacrophage $F(ab')_2$ upon attachment and ingestion mediated by the Fc receptors and by the complement receptors of nonactivated macrophages. Antimacrophage IgG efficiently blocked binding and ingestion of $E(IgG)$ by macrophages, but had no inhibitory effect upon the uptake of latex particles by these cells (data for latex particles not shown). The Fc receptor blocking activity of antimacrophage IgG was dependent upon the presence of the Fc portion of the IgG molecule; $F(ab')_2$ fragments of the same immunoglobulin did not inhibit binding or ingestion of $E(IgG)$ by macrophages. These results are identical to those of Holland et al. (19). Pretreatment of macrophages with an amount of antimacrophage IgG sufficient to block completely Fc receptor-mediated binding and ingestion of

TABLE I
Cytotoxic Assay of Antimacrophage IgG and Antimacrophage F(ab')₂

Macrophage pretreatment	Antimacrophage immunoglobulin	Complement*	Lysis‡
			%
None	IgG	+	>95
None	IgG	-	<5
None	F(ab') ₂	+	<5
None	F(ab') ₂ + SAR IgG	+	>95
None	SAR IgG	+	<5
Trypsin	IgG	+	>95
Trypsin	F(ab') ₂	+	<5

* Fresh rabbit serum diluted 1:20 in medium 199.

‡ Determined by morphology and by trypan blue dye exclusion.

TABLE II
Influence of Antimacrophage IgG and of Trypsin Treatment of Nonactivated Macrophages on the Attachment and Ingestion of Sensitized Erythrocytes

Erythrocyte complex	Pretreatment of macrophages	24-h explanted macrophages				48-h explanted macrophages			
		Attachment*		Ingestion		Attachment*		Ingestion	
		%	Index	%	Index	%	Index	%	Index
E(IgG)	None	100	746	88	602	100	1620	97	1,576
	Antimacrophage IgG	21	51	0	0	16	98	3	3
	Antimacrophage F(ab') ₂	100	786	92	736	ND	ND	ND	ND
	Trypsin	100	773	91	663	100	1,005	98	980
E(IgG)C	None	100	928	90	786	100	1,474	95	1,394
	Antimacrophage IgG	95	1,049	1	1	91	1,200	8	8
	Antimacrophage F(ab') ₂	96	960	96	672	ND	ND	ND	ND
	Trypsin	100	780	96	720	100	1,000	96	960
E(IgM)C	None	96	1,060	19	38	90	1,170	6	20
	Antimacrophage IgG	91	892	0	0	91	939	2	2
	Trypsin	11	15	2	2	0	0	1	1
E		1	1	0	0	2	4	0	0
E(IgM)		1	2	1	1	9	30	4	9

* Percent attachment and attachment index include figures for attachment plus ingestion. In other experiments, attachment was evaluated separately by incubating macrophages with the various erythrocyte preparations under conditions in which ingestion was inhibited (4°C or in the presence of NaF at 37°C). The results obtained for attachment were not significantly different from those presented here.

E(IgG) had no inhibitory effect upon the binding of E(IgG)C or E(IgM)C to the macrophage surface. Under these conditions, E(IgG)C and E(IgM)C were bound to the macrophage surface presumably by complement receptors; however, no ingestion of E(IgG)C or E(IgM)C occurred. Thus, antimacrophage IgG blocked specifically Fc receptor-mediated erythrocyte binding and ingestion but had no effect upon binding mediated by the macrophage complement receptors.

Failure of Antimacrophage IgG to Block Complement-Mediated Ingestion by Activated Macrophages. It was possible that phagocytosis of E(IgM)C by activated macrophages was due to an enhanced capacity of these cells to

recognize small quantities of IgG contaminating the anti-E IgM fraction used to prepare E(IgM)C. The following experiments rule out this possibility. (a) The anti-E IgM preparation used did not contain detectable quantities of IgG, as assayed by the inability of antibody directed against the Fc fragment of IgG to agglutinate E(IgM) or E(IgM)C. (b) Antimacrophage IgG blocked almost completely the ingestion of E(IgG) by both 24-h and 48-h explanted activated macrophages (Table III). However, it only partially blocked the uptake of E(IgM)C and E(IgG)C by 24-h explanted cells and had only a minimal inhibitory effect upon phagocytosis of E(IgM)C and E(IgG)C by 48-h explanted macrophages. Ingestion of E(IgG)C by 24-h and 48-h explanted nonactivated macrophages was strongly inhibited by this antimacrophage IgG (Table II). These results confirm that phagocytosis of E(IgM)C by activated macrophages is mediated by complement receptors, and not by Fc receptors, of the macrophage plasma membrane.

Effect of Trypsin upon Complement-Mediated Attachment and Ingestion. Incubation of nonactivated macrophages with 1 mg/ml of trypsin for 30 min at 37°C abolished binding of E(IgM)C to the macrophage complement receptors without detectable changes in macrophage binding and ingestion of E(IgG) and E(IgG)C (Table II). When 24-h or 48-h explanted activated macrophages were trypsinized, they neither bound nor ingested E(IgM)C. The identical trypsin sensitivity of complement receptors of activated and of nonactivated macrophages further supports our contention that the ingestion of E(IgM)C by activated macrophages is mediated by the complement receptor.

TABLE III
Ingestion of Sensitized Erythrocytes by Untreated and by Antimacrophage IgG-Treated Activated Macrophages

Erythrocyte complex	Pretreatment with antimacrophage IgG	Ingestion by macrophages			
		24-h explanted		48-h explanted	
		%	Index	%	Index
E(IgG)	—	82	1,412	100	2,280
	+	8	11	28	62
E(IgG)C	—	100	1,840*	100	2,260
	+	71	363*	96	1,571
E(IgM)C	—	66	518	89	986
	+	43	219	84	710
E	—	8	8	9	24
E(IgM)	—	14	49	26	65

* It should be noted that ingestion of E(IgG)C by untreated activated macrophages occurs via both Fc receptors and by complement receptors. The drop in ingestion index of 24 h explanted cells from 1,840 to 363 is due chiefly to blocking of the participation of Fc receptors by antimacrophage IgG. The figure of 363 should be compared with the ingestion index of 518 for E(IgM)C when considering blocking of complement receptors by antimacrophage IgG.

Discussion

The *in vitro* cultivation of macrophages from the unstimulated mouse peritoneal cavity results in marked enhancement of the capacities of these cells to phagocytize IgG-coated erythrocytes. Nearly twice as many E(IgG) were ingested by 48-h explanted nonactivated macrophages as by nonactivated cells explanted for only 24 h (Fig. 2). Thioglycollate-induced (activated) macrophages are 1.5–2 times as efficient as nonactivated macrophages in ingesting E(IgG), and their capacity to phagocytize E(IgG) also increases with *in vitro* cultivation (Fig. 2 *a* and *b*). Since we have not measured the time required for the ingestion of individual erythrocytes by activated and by nonactivated macrophages, we cannot be certain whether activated cells merely have an increased total phagocytic capacity or whether they exhibit an increased rate of ingestion as well.

Trypsin treatment of macrophages abolishes complement receptor binding activity (2) but does not inhibit the capacity of these cells to phagocytize E(IgG)C. Conversely, macrophages incubated with antimacrophage IgG [an antibody preparation which specifically inhibits Fc receptor activity (Table II and ref. 19)], bind but do not ingest E(IgG)C. Thus the phagocytosis of E(IgG)C by nonactivated macrophages is mediated by the Fc receptor.

Erythrocytes coated with IgM are neither bound nor ingested by macrophages; erythrocytes coated with IgM and complement are bound by nonactivated macrophages but are not ingested by them (Fig. 1, Table II). These results, and those described above using antimacrophage IgG-treated macrophages, confirm and extend the findings of Lay and Nussenzweig (2) and of Mantovani et al. (3). These latter authors showed that E(IgG)C incubated with F(ab) anti-IgG are bound to, but not ingested by, mouse peritoneal macrophages. Thus, the complement receptors of nonactivated mouse peritoneal macrophages do not mediate erythrophagocytosis.

Macrophages induced by the intraperitoneal injection of thioglycollate medium phagocytize erythrocytes coated with IgM and complement [E(IgM)C] (Fig. 3). In this respect they differ qualitatively from nonactivated mouse macrophages. That the ingestion of E(IgM)C is mediated by C3b receptors on the plasma membrane of activated macrophages was confirmed as follows: (*a*) E(IgM) were neither bound nor ingested by activated macrophages. (*b*) The Fc receptor blockade mediated by antimacrophage IgG did not interfere significantly with the ingestion of either E(IgM)C or E(IgG)C. (*c*) Appropriate trypsin treatment of activated macrophages abolished ingestion of E(IgM)C without affecting the uptake of E(IgG) or E(IgG)C. Consequently, ingestion is not due to an increased ability of activated macrophages to recognize the Fc portion of IgG or IgM.

Activated macrophages are more efficient in phagocytizing damaged erythrocytes. It was possible that complement fixation, even if not complete under the conditions used, produced alterations in the erythrocyte membrane which could be recognized by activated macrophages. To rule out this possibility, we (5) further incubated E(IgM)C with fresh AKR mouse serum after complement fixation. Under these conditions, C3b is converted to C3c and C3d; C3d is inactive as a ligand for macrophage complement receptors. These C3d-coated eryth-

rocytes were neither bound to nor ingested by activated macrophages. We⁴ also incubated C3b-coated erythrocytes with a low concentration of trypsin at 37°C or with a higher concentration at 4°C. Under these conditions as well, C3b is rendered inactive as a ligand for macrophage C3b receptors, and the trypsin-treated erythrocytes are neither bound nor ingested. For these reasons, it seems unlikely that phagocytosis of E(IgM)C by activated macrophages is secondary to complement-induced damage of the red cell surface.

C3b inactivator (KAF, congenitally activating factor) is a heat stable enzyme present in normal serum which cleaves particle-bound C3b into a C3c fragment which is released into the fluid phase and a C3d fragment which remains bound to the particle (reviewed in 24). Many leukocytes bear receptors for C3, and the interaction of antigen-antibody complexes with one or another form of C3 has physiological importance in determining the fate of these complexes. B lymphocytes have receptors for both C3b and C3d-bearing erythrocytes (25, 26), while guinea pig (27) and human (25) polymorphonuclear leukocytes, rabbit and human alveolar macrophages (6), and activated and nonactivated mouse peritoneal macrophages (5) all have membrane receptors for C3b only. Of these, only guinea pig and human polymorphonuclear leukocytes (25,27) and activated mouse peritoneal macrophages phagocytize via their complement receptors.

The mechanism responsible for the altered function of the complement receptors of activated macrophages is not understood. The C3-binding activities of C3 receptors on activated and nonactivated macrophages exhibit similar trypsin sensitivities and requirements for the C3b fragment of the third complement component. Thus, aside from differences in their functional capacities, there is no reason to assume that the C3-binding activities of activated and nonactivated macrophages are mediated by different membrane receptors. It is possible that C3 receptors of activated macrophages are linked to contractile elements in the macrophage cytoplasm, whereas C3 receptors of nonactivated cells are not. It is also possible that the plasma membranes of activated and of nonactivated macrophages contain different concentrations of C3 receptor molecules and that macrophages containing fewer than a threshold number of receptors will bind but will not ingest complement-coated particles. These two possibilities are not mutually exclusive.

The capacity of activated macrophages to phagocytize complement-coated erythrocytes, and, by analogy, other complement-coated particles, raises several issues relative to the *in vivo* significance of these findings. Particles coated with early antibody (IgM) and exposed to plasma complement factors may be bound but should not be ingested by nonactivated mouse peritoneal macrophages. Inflammatory stimuli which cause macrophage activation will produce alterations in the functional activity of the macrophage complement receptors leading to the phagocytosis of complement-coated particles. In fact, the capacity of activated macrophages to ingest C3b-coated particles provides an explanation at the cellular level for the *in vivo* observations of Atkinson and Frank (28). They showed that IgM-coated homologous erythrocytes injected intravenously into

⁴Griffin, F.M., J.A. Griffin, J.E. Leider, and S.C. Silverstein. Manuscript in preparation.

normal guinea pigs were briefly sequestered by the liver [presumably as E(IgM)C3b] and then released back into the circulation [presumably as E(IgM)C3d]. The half-life of these erythrocytes was nearly the same as that of nonopsonized erythrocytes. On the other hand, IgM-coated erythrocytes injected intravenously into guinea pigs infected with BCG, a bacterium known for its capacity to cause macrophage activation, were localized to the liver and were presumably destroyed there. It is likely that these erythrocytes [E(IgM)C3b] were ingested by the activated hepatic macrophages of these BCG-infected animals. That early acting complement components were necessary for hepatic localization of IgM-coated erythrocytes to occur was demonstrated in studies with guinea pigs congenitally deficient in the fourth component of complement. In these animals, no erythrocyte trapping occurred (29).

The observed association between activated macrophages and the phagocytic capacity of their C3 receptors may be more than a fortuitous one in the handling of obligatory intracellular parasites, such as *Listeria monocytogenes* and *Brucella abortus*, which replicate efficiently in nonactivated macrophages but are killed within activated macrophages (7). Coating of these organisms with IgM and complement might enhance their clearance by activated macrophages.

We do not know whether the activation of macrophages induced by the intraperitoneal injection of thioglycollate medium is identical to the macrophage activation described by Mackaness (7) and others (8-13) in chronic infections and delayed hypersensitivity reactions. Preliminary results indicate that intraperitoneal injection of BCG or endotoxin elicit macrophages which also phagocytize via their C3 receptors. Macrophages elicited by these agents exhibit a lower intensity of C3 receptor-mediated phagocytosis than do cells elicited with thioglycollate medium. Although the reasons for these differences are not yet apparent, it is clear that macrophage activation can be achieved by a number of agents and that macrophages exist in a variety of states or levels of activation. The identification of markers for each of these states is a necessary prerequisite for understanding the mechanism(s) underlying their induction and the role(s) they play in macrophage physiology. In this context we propose that ingestion via the C3b receptor is a simple, quick, and quantitative test for the identification of activated macrophages.

Summary

We have examined the roles of Fc receptors and complement receptors in mediating the interaction of sensitized sheep erythrocytes (E) with activated and with nonactivated mouse peritoneal macrophages. Both activated and nonactivated macrophages ingest IgG-coated erythrocytes [E(IgG)]; activated cells ingest 1.5-2 times as many E(IgG) as do nonactivated macrophages. Thus, there is a quantitative difference in Fc receptor-mediated ingestion between activated and nonactivated macrophages.

There is, however, a qualitative difference in function of complement receptors of activated and nonactivated macrophages. Nonactivated macrophages avidly bind complement-coated E [E(IgM)C], but do not ingest them to a significant degree. Activated macrophages, on the other hand, bind and ingest E(IgM)C. The possibility of Fc receptor participation in mediating ingestion of E(IgM)C

by activated macrophages was eliminated by blocking Fc receptors with an antimacrophage IgG fraction. Activated macrophages treated with antimacrophage IgG did not ingest E(IgG) but did ingest both E(IgM)C and E(IgG)C. Nonactivated macrophages treated with antimacrophage IgG did not interact at all with E(IgG). These cells bound, but did not ingest, E(IgG)C and E(IgM)C. Complement receptor-mediated ingestion is a marker for macrophage activation and may be physiologically important in the elimination of complement-coated particles.

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