EFFECTS OF SOLUBLE IMMUNE COMPLEXES ON Fc RECEPTOR- AND C3b RECEPTOR-MEDIATED PHAGOCYTOSIS BY MACROPHAGES*

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Work from this laboratory has previously demonstrated that the function of the complement receptor of the resident mouse peritoneal macrophage can be converted in vitro from mediating only particle binding to promoting both the binding and ingestion of complement-coated erythrocytes (1, 2). Enhanced complement receptor function is imparted to macrophages by a low molecular weight lymphokine, the generation and elaboration of which require a unique series of cellular interactions (1). Macrophages must first phagocytize IgG-containing immune complexes; these cells thereby acquire the ability to trigger, by a contact-dependent mechanism, T lymphocytes to elaborate the product that augments the complement receptor function of freshly explanted macrophages. The effect of the lymphokine upon macrophages' phagocytic capability is selective and specific for the complement receptor, for lymphokine-treated macrophages do not indiscriminately interiorize all particles bound to their plasma membranes and do not more avidly, more efficiently, or more rapidly phagocytize IgG-coated particles via their Fc receptors (2).

All of the cellular and molecular components—macrophages, T lymphocytes, antigen, antibody, immune complexes, and complement components—necessary for both the generation of the lymphokine and the expression of its activity are present at sites of immunologically mediated inflammation. We previously suggested that this lymphokine may be important in a variety of infectious, inflammatory, and neoplastic disorders and proposed a model by which the appropriate cellular and molecular interactions might occur in vivo (1). Briefly, microorganisms and tumor cells become coated with both IgG and C3b in the immune host. These offenders also shed antigens that become bound by host antibody. Macrophages present at sites of immunologically mediated inflammation are, therefore, exposed to large quantities of immune complexes composed of IgG and either microbial antigens or neoantigens from tumor cells. We proposed that ingestion of these complexes might block or saturate the cells' Fc receptors so that macrophages would be unable to phagocytize via these receptors the offenders they were invited to destroy. In their normal physiologic state, macrophages could bind the microbe or tumor cell via their C3b receptors but could not ingest it and would probably release it when C3b inactivator cleaved C3b; the offender would be unharmed after such an encounter (3, 4). However, ingestion of

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immune complexes would permit macrophages to interact with T lymphocytes, thereby triggering the lymphocytes to elaborate the lymphokine which enhances macrophages' complement receptor function. Macrophages would then be able to ingest and to destroy the invaders via their complement receptors.

For our model to have pathophysiologic relevance, several conditions must be satisfied. First, ingestion of soluble immune complexes must result in severe impairment of macrophage Fc receptor function. Second, ingestion of these complexes must not impair the macrophages' interaction with particles by other means, especially via their complement receptors. Third, macrophages that have ingested immune complexes must be able to respond to the lymphokine and to phagocytize complement-coated particles. Results of experiments reported in this paper satisfy these conditions and provide strong evidence in support of the proposed model.

Materials and Methods

Reagents and Media. Glutaraldehyde, 50% aqueous solution (Fisher Scientific Co., Pittsburgh, Pa.); sheep erythrocytes (E)[1] (Animal Blood Center, Syracuse, N. Y.); fetal bovine serum (FBS) (Grand Island Biological Co., Grand Island, N. Y.); trypsin (lot T8253, twice recrystallized; Sigma Chemical Co., St. Louis, Mo.); ovomucoid trypsin inhibitor (Worthington Biochemical Corp., Freehold, N. J.); and Medium 199 (Microbiological Associates, Walkersville, Md.) were obtained from the manufacturers indicated. FBS was decomplemented by heating at 56°C for 30 min before use. Lyophilized human serum albumin (HSA; recrystallized five times; Sigma Chemical Co.) was reconstituted in phosphate-buffered saline (PBS) at a concentration of 20 mg/ml. Lyophilized rabbit gamma globulin (N. L. Cappel Laboratories Inc., Cochranville, Pa.) was reconstituted in PBS at a concentration of 10 mg/ml; a portion of the reconsitituted material was aggregated by heating at 63°C for 45 min (5). A 0.1-ml aliquot containing 0.5 mg of aggregated IgG was incubated with 0.1 ml of fresh mouse serum for 10 min at 37°C to prepare complement-treated IgG aggregates.

Antibody Preparations. Rabbit anti-sheep E IgG and rabbit anti-sheep E IgM were obtained from Cordis Laboratories Inc. (Miami, Fla.). Rabbit anti-ox E IgG was a gift from Dr. E. Pearl, University of Alabama in Birmingham, Birmingham, Ala. Rabbit anti-HSA antiserum was prepared by repeatedly immunizing a rabbit with HSA; it was heat decomplemented (56°C, 30 min) before use. Rhodamine-labeled HSA, rhodamine-labeled rabbit IgG, and rhodamine-labeled Fab fragments of rabbit IgG were gifts from Dr. S. Jackson, University of Alabama in Birmingham, Birmingham, Ala.; portions of the IgG and Fab preparations were aggregated by heating at 63°C for 45 min.

Immunologically Coated Erythrocytes. E, E coated with anti-E IgM (E[IgM]), E(IgM) coated with the first four complement components (E[IgM]C), E coated with anti-E IgG (E[IgG]), and E(IgG) coated with the first four complement components (E[IgG]C) were prepared as previously described (4, 6). Some E(IgG) were prepared using smaller quantities of anti-E IgG. E(IgM)C-IgG were prepared by incubating E(IgM)C with 1 μl/ml of anti-E IgG for 15 min at 37°C. Ox erythrocytes (ox E) coated with anti-ox E IgG (oxE[IgG]) were prepared as previously described (1).

Preparation of Soluble Immune Complexes. 1 ml of anti-HSA antiserum was incubated with 10 mg of HSA, some aliquots of which were rhodamine-labeled for 1 h at 37°C. Although the exact antigen-antibody ratio cannot be determined, the mixture contained an excess of antigen, the estimated molar ratio being at least 10:1, assuming 25% of the IgG in the antiserum was specific for HSA. No precipitates of a size sufficient to cloud the solution were formed. Sham immune complexes were prepared exactly as HSA-anti-HSA complexes, but using nonimmune rabbit serum in place of immune serum.

[1] *Abbreviations used in this paper:* AI, attachment index; 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; E(IgM)C-IgG, E(IgM)C coated with anti-E IgG; FBS, fetal bovine serum; HSA, human serum albumin; ox E, ox erythrocytes; oxE(IgG), ox E coated with anti-ox E IgG; PBS, phosphate-buffered saline; PI, phagocytic index.

Complement-treated immune complexes were prepared by incubating 0.1 ml of HSA-anti-HSA complexes containing 0.17 mg of HSA with 0.1 ml of fresh mouse serum for 10 min at 37°C.

Animals and Cells. 20- to 30-g female mice (strain CD-1) were obtained from Charles River Breeding Laboratories, Inc., Wilmington, Mass. They served as sources of peritoneal macrophages and thymocytes, which were cultivated and used as previously described (1).

Fluorescence Microscopy. Macrophage cultures that had been incubated with rhodamine-labeled material were fixed with glutaraldehyde and examined with a Leitz Orthoplan fluorescence microscope (E. Leitz, Inc., Rockleigh, N. J.) equipped with a C5 100 W/2 mercury vapor lamp (Philips Electronic Instruments, Inc., Mahwah, N. J.), a Ploem epi-illumination system, dichroic mirrors, and filters selective for rhodamine (7).

Preparation of Supernates Containing the Lymphokine That Augments Macrophage Complement Receptor Function. Supernatant media were prepared as described previously (1). Briefly, monolayers containing 1×10^6 mouse peritoneal macrophages were overlaid with fresh medium without FBS; either 0.2 ml of 0.5% oxE(IgG) or HSA-anti-HSA complexes containing 1 mg of HSA were added to each monolayer and incubation continued for 4 h at 37°C. Cultures were then washed and noningested erythrocytes lysed with NH_4Cl where appropriate. 5×10^6 thymocytes in medium were added to each dish and incubation continued for 24 h at 37°C. Supernatant medium from each culture was harvested, centrifuged at 750 *g* for 10 min to remove cells and cellular debris, and assayed for its effect upon macrophage complement receptor function, as described below. There was no substantial difference in lymphokine activity between supernates prepared using oxE(IgG) and those prepared using HSA-anti-HSA complexes as the material fed to macrophages. Results are therefore presented without regard to which was used.

Assessment of Lymphokine Activity. Lymphokine activity was assessed in two ways. The first, which will be referred to as the preincubation assay, was performed as previously described (1). Freshly established macrophage monolayers were covered with experimental supernates and incubated at 37°C for 48 h; supernates were removed and monolayers covered with fresh medium. The complement receptor function of these macrophages was assessed by adding 0.2 ml of 0.5% E(IgM)C, incubating the cultures for 30 min at 37°C, and determining the fate of the E(IgM)C by phase contrast microscopy.

The second method, which will be referred to as the direct assay, was performed as previously described (2). 48-h explanted resident mouse peritoneal macrophages, which had been maintained in medium-20% FBS, were washed and overlaid with supernates. E(IgM)C were added to cultures simultaneously with supernates, incubation continued for 30 min at 37°C, and the fate of E(IgM)C was determined microscopically.

The effect of the lymphokine upon Fc receptor-mediated and nonimmunologically mediated phagocytosis was assessed exactly as described above, using E(IgG) and zymosan, respectively, in place of E(IgM)C.

Phase Contrast Microscopy. Cover slip cultures were fixed with glutaraldehyde and examined by phase contrast microscopy as previously described (1).

Miscellaneous. Protein concentrations were determined by the method of Lowry et al. (8), using bovine serum albumin as a standard. Cell viability was assessed by determining the ability of cells to exclude 0.4% trypan blue dye in 0.15 M NaCl after 10 min at 25°C. Some macrophages were treated with trypsin as previously described (4).

Presentation of Results. Each result given represents the average of at least three separate determinations, each performed in duplicate. Attachment index (AI) and phagocytic index (PI) are the number of erythrocytes attached or ingested by 100 macrophages and were obtained by multiplying the percent of macrophages that had attached or ingested any erythrocytes by the average number of erythrocytes attached or ingested per macrophage. Each result is presented as mean ± SEM.

Results

Ingestion of Soluble Immune Complexes and Aggregated IgG by Macrophages. To ensure that HSA-anti-HSA complexes and aggregated IgG were ingested by macrophages

and to define the structure and physical state of IgG required for its uptake, the fate of rhodamine-labeled IgG and complexes was determined microscopically.

Macrophages were incubated for 1 h at 37°C with either of the following: HSA-anti-HSA complexes prepared with rhodamine-labeled HSA; sham complexes, a mixture of rhodamine-labeled HSA and nonimmune rabbit serum; rhodamine-labeled rabbit IgG; rhodamine-labeled, heat-aggregated rabbit IgG; or rhodamine-labeled, heat-aggregated Fab fragments of rabbit IgG.

Bright field microscopy revealed that macrophages that had been incubated with either HSA-anti-HSA complexes or aggregated IgG contained numerous pinocytic vacuoles, whereas macrophages that had been incubated with any of the other three preparations did not. These results suggested that only immune complexes and aggregated IgG were ingested by macrophages.

This suggestion was confirmed by fluorescence microscopy. 97 and 83% of macrophages that had been incubated with either rhodamine-labeled HSA-anti-HSA complexes or rhodamine-labeled aggregated IgG, respectively, demonstrated bright red intracytoplasmic, granular fluorescence, whereas only a small percentage of macrophages that had been incubated with any of the control preparations contained rhodamine-labeled material (Table I). Thus, immune complexes and aggregated IgG were avidly ingested by macrophages, uptake of IgG required that it be either aggregated or bound to antigen, and uptake occurred via the macrophages' Fc receptors.

Effect of Ingestion of Soluble Immune Complexes upon Macrophages' Ability to Ingest IgG-coated Particles. The next experiments were designed to determine whether or not soluble immune complexes and aggregates could block or saturate macrophage Fc receptors so that the cell's ability to phagocytize IgG-coated particles was impaired. Macrophages were incubated for 1 h at 37°C with varying concentrations of either soluble HSA-anti-HSA complexes or heat-aggregated rabbit IgG, which behaves similarly in many respects to immune complexes (5, 9). Cultures were then washed and incubated for 30 min at 37°C with E(IgG) that had been prepared using varying concentrations of anti-E IgG. Nonaggregated IgG, anti-HSA antiserum alone, and sham complexes, a mixture containing both HSA and nonimmune rabbit serum, served as controls. As indicated in Fig. 1, previous uptake of either immune complexes or aggregated IgG by macrophages impaired, in a dose-dependent manner, the cells' ability to ingest E(IgG). Macrophages that had not ingested complexes efficiently

TABLE I

Ingestion of Soluble Immune Complexes and Aggregated IgG by Macrophages

Macrophages incubated with:	Macrophages containing rhodamine label
	%
HSA*-anti-HSA	97
HSA*-nonimmune serum‡	6
Nonaggregated rabbit IgG*	10
Aggregated rabbit IgG*	83
Aggregated Fab fragments* of IgG	18

* Rhodamine labeled.

‡ Sham immune complexes.

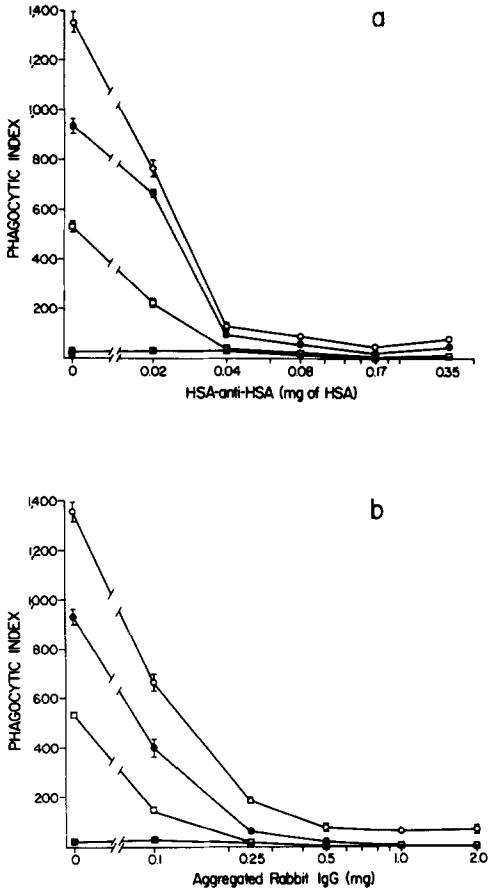

FIG. 1. Effect of ingestion of soluble immune complexes and aggregated rabbit IgG upon macrophages' ability to ingest E(IgG). Macrophages were preincubated for 1 h at 37°C with either HSA-anti-HSA complexes, the HSA contents of which are indicated on the abscissa (a), or the quantities of aggregated IgG indicated on the abscissa (b). These macrophages were then incubated for 30 min at 37°C with E(IgG) prepared with 3 (○), 1 (●), 0.5 (□), or 0.1 (■) μl/ml of anti-E IgG and the PI (ordinate) determined.

ingested E(IgG) prepared with 1 μl/ml or more anti-E IgG but ingested E(IgG) prepared with smaller quantities of antibody less efficiently. Inhibition of phagocytosis of E(IgG) was dependent upon both the concentration of IgG in complexes or aggregates and the concentration of IgG used to opsonize erythrocytes (Fig. 1). None of the control preparations affected the ability of macrophages to phagocytize E(IgG) (data not shown).

In all subsequent experiments, I prepared E(IgG) with 1 μl/ml of anti-E IgG, a concentration that yielded nearly maximal ingestion of E(IgG) by normal macrophages, and used 0.17 mg (HSA concentration) of HSA-anti-HSA and 0.5 mg of aggregated IgG per culture, concentrations that inhibited the uptake of these E(IgG) by >95%.

Effect of Ingestion of Soluble Immune Complexes upon Macrophages' Ability to Bind E(IgG). Whereas ingestion of aggregates and complexes strikingly impaired macrophages' ability to ingest E(IgG) in the above experiments, there was no impairment

of their ability to bind E(IgG) (Table II, column 1). Macrophages possess two classes of Fc receptors: a trypsin-sensitive receptor that recognizes mouse IgG_{2a} and a trypsin-resistant receptor that recognizes mouse IgG1 and IgG_{2b} (10). In addition to their differences in immunoglobulin subclass recognition, these receptors may mediate different functions, the trypsin-sensitive receptor promoting only particle binding, the trypsin-resistant receptor promoting both particle binding and ingestion (11). Because particles coated with rabbit IgG may bind to both classes of receptor, it was possible that aggregates and immune complexes nearly completely blocked the function of one class but had little or no effect on the other.

To test this possibility, macrophages were fed aggregated IgG or HSA-anti-HSA complexes, washed, subjected to trypsin treatment, then incubated with E(IgG) for 30 min at 37°C. Attachment of E(IgG) by these macrophages was markedly lower than attachment by companion macrophages that had not been trypsin-treated (Table II, lines 2 and 3). As we have previously shown (4), trypsin treatment had no effect on the ability of normal macrophages to bind E(IgG) (Table II, line 1). These results indicate that residual attachment of E(IgG) by macrophages that had ingested immune complexes was mediated by trypsin-sensitive Fc receptors and that nearly all trypsin-resistant Fc receptor activity was blocked by the ingestion of immune complexes. Because the E(IgG) bound to macrophages that had ingested immune complexes were not phagocytized, these results also support the hypothesis that trypsin-sensitive Fc receptors promote binding but not ingestion of IgG-coated particles.

Effect of Ingestion of Soluble Immune Complexes upon Macrophages' Ability to Bind and Ingest Particles by Means other than Their Fc Receptors. Macrophages, some of which had ingested either aggregated IgG or HSA-anti-HSA complexes, were incubated for 30 min at 37°C with either zymosan, a particle which is ingested by nonimmunologic means, or E(IgM)C, a particle whose interaction with macrophages is governed by the cells' complement receptors. Macrophages that had previously ingested either aggregated IgG or immune complexes bound E(IgM)C and phagocytized zymosan as effectively as nonpretreated macrophages (Table III). Even using maximum noncytotoxic quantities of aggregates and complexes, I was unable to find a concentration that impaired macrophages' ability to interact with particles by means other than their Fc receptors (data not shown).

These results indicate that the ingestion of soluble immune complexes selectively blocks Fc receptor-mediated phagocytosis but does not alter the interaction of macrophages with particles by other immunologic or nonimmunologic mechanisms.

Effect of Ingestion of Soluble Immune Complexes upon the Ability of Lymphokine-treated Macrophages to Phagocytize Various Particles. Some macrophage cultures were preincu-

TABLE II

Effect of Ingestion of Soluble Immune Complexes upon Macrophages' Ability to Bind E(IgG)

Macrophages preincubated with:	AI of E(IgG) by macrophages that were	
	Nontreated	Trypsin-treated
—	1,465 ± 12	1,470 ± 11
HSA-anti-HSA	1,425 ± 11	172 ± 78
Aggregated IgG	1,420 ± 6	55 ± 5

TABLE III

Effect of Ingestion of Soluble Immune Complexes upon Macrophages' Ability to Bind and Ingest Particles by Means Other than Their Fc Receptors

Macrophages preincubated with:	AI of E(IgM)C	PI of zymosan
—	1,078 ± 122	529 ± 16
HSA-anti-HSA	1,063 ± 197	497 ± 28
Aggregated IgG	1,055 ± 155	480 ± 47

TABLE IV

Effect of Ingestion of Soluble Immune Complexes upon the Ability of Lymphokine-treated Macrophages to Ingest Various Particles

Macrophages preincubated with:	Particle presented	PI of particle by macrophages		
		Not treated with lymphokine	Treated with lymphokine	
			By preincubation	Directly
—	E(IgM)C	5 ± 2	278 ± 59	226 ± 16
HSA-anti-HSA	E(IgM)C	5 ± 4	236 ± 10	225 ± 26
Aggregated IgG	E(IgM)C	4 ± 3	291 ± 25	218 ± 13
—	E(IgG)	1,114 ± 48	1,148 ± 43	979 ± 100
HSA-anti-HSA	E(IgG)	20 ± 4	31 ± 6	32 ± 12
Aggregated IgG	E(IgG)	24 ± 3	28 ± 9	27 ± 4
—	Zymosan	529 ± 16	469 ± 19	504 ± 21
HSA-anti-HSA	Zymosan	497 ± 28	498 ± 18	498 ± 17
Aggregated IgG	Zymosan	480 ± 47	474 ± 18	508 ± 25

bated in supernates containing the lymphokine that imparts to macrophages the ability to phagocytize via their complement receptors. Cultures were washed, overlaid with fresh medium containing either aggregated IgG or HSA-anti-HSA complexes, incubated for 1 h at 37°C, washed, and incubated for 30 min at 37°C with E(IgM)C (preincubation assay). Other cultures were incubated for 1 h at 37°C with either aggregated IgG or HSA-anti-HSA complexes, washed, overlaid with supernates and E(IgM)C simultaneously, and incubated for 30 min at 37°C (direct assay). Erythrocyte attachment and ingestion were scored microscopically.

All macrophages avidly bound E(IgM)C (data not shown). The results of phagocytosis assays are presented in Table IV, lines 1–3. Like normal macrophages, macrophages which had been preincubated with either aggregated IgG or HSA-anti-HSA complexes did not ingest E(IgM)C (Table IV, column 1, lines 1–3), indicating that the ingestion of complexes does not trigger macrophages to ingest particles bound to their complement receptors, a result consistent with our previous finding that triggering one type of phagocytic receptor does not trigger other receptors that have the potential to phagocytize (4, 12).

Macrophages that had been treated with lymphokine-containing supernates, either by preincubation or at the time of assay, efficiently phagocytized E(IgM)C, with PI of 278 and 226, respectively (Table IV, line 1, columns 2 and 3). Macrophages that

ingested IgG aggregates or complexes either after preincubation in supernates (preincubation assay) (Table IV, column 2, lines 2 and 3) or before exposure to supernates (direct assay) (Table IV, column 3, lines 2 and 3) ingested E(IgM)C as efficiently as macrophages which had not ingested complexes.

In parallel cultures, aggregate- and complex-treated macrophages ingested zymosan particles normally but were unable to ingest E(IgG) (Table IV, column 1, lines 4–9). Supernate treatment did not enhance the ability of aggregate- or complex-treated macrophages to ingest E(IgG) (Table IV, columns 2 and 3, lines 4–6), confirming our previous finding that supernates do not augment Fc receptor function (2). Neither did supernate treatment enhance the ability of macrophages to ingest zymosan (Table IV, columns 2 and 3, lines 7–9), demonstrating that supernates do not augment nonimmunologically mediated phagocytosis.

These results indicate that ingestion of immune complexes by macrophages does not impair either the macrophages' ability to respond to supernates or the ability of supernate-treated macrophages to phagocytize via their complement receptors.

Effect of Ingestion of Soluble Immune Complexes upon Macrophages' Ability to Ingest Particles Coated with Both IgG and C3b. At sites of immunologically mediated inflammation in an immune host, the target to be attacked is coated with both IgG and C3b. Macrophage complement receptors very efficiently promote binding of C3b-coated particles, and the interaction of macrophage Fc receptors with particle-bound IgG is greatly strengthened when the particle is coated with both ligands and bound by both receptors (13). Immune complexes may therefore have been unable to block Fc receptors sufficiently well to prevent phagocytosis of particles coated with both IgG and C3b. To test this possibility, I determined the effect of ingestion of soluble immune complexes upon the ability of supernate-treated and nontreated macrophages to interact with particles coated with both immunologic ligands.

Macrophages were fed soluble IgG-containing complexes or aggregates, as in the experiments described above. Some cultures were treated with supernates, either by preincubation or during the assay culture, also as described above, and the interaction of the various macrophage preparations with erythrocytes coated with both IgG and C3b was assessed.

Erythrocytes coated with both IgG and C3b—E(IgG)C and E(IgM)C-IgG—were prepared as described in Materials and Methods. Both E(IgG)C and E(IgM)C-IgG were used for two reasons. First, in some circumstances, complement fixation by antigen-antibody complexes results in the deposition of complement component(s) on or near the Fc portion of the immunoglobulin (14, 15), thereby preventing the interaction of the immunoglobulin with macrophage Fc receptors (15). Thus, had macrophages failed to ingest E(IgG)C, the result might have been due to nonavailability of the Fc portion of IgG to the cells' Fc receptors. Second, in order for C3- and Fc-receptor cooperation to occur, it might be important for IgG and C3b to be in close proximity on the particle surface. Thus, had macrophages failed to ingest (E(IgM)C-IgG, the result might have been due to inappropriate distribution of the two ligands.

Normal macrophages avidly ingested erythrocytes coated with both ligands (Table V, column 1, lines 1 and 4). Previous ingestion of IgG-containing aggregates or complexes had no effect upon the ability of macrophages to bind these erythrocytes (data not shown) but markedly impaired the cells' ability to ingest them (Table V,

TABLE V

Effect of Ingestion of Soluble Immune Complexes upon Macrophages' Ability to Ingest Particles Coated with Both IgG and C3b

Macrophage preincubated with:	Particle presented	PI of E coated with both IgG and C3b by macrophages		
		Not treated with lymphokine	Treated with lymphokine	
			By preincubation	Directly
—	E(IgG)C	1,405 ± 27	1,335 ± 21	1,320 ± 27
HSA-anti-HSA	E(IgG)C	46 ± 3	320 ± 35	295 ± 43
Aggregated IgG	E(IgG)C	27 ± 10	307 ± 58	252 ± 20
—	E(IgM)C-IgG	1,191 ± 38	1,066 ± 35	1,029 ± 36
HSA-anti-HSA	E(IgM)C-IgG	42 ± 11	402 ± 21	250 ± 30
Aggregated IgG	E(IgM)C-IgG	62 ± 14	464 ± 77	364 ± 30

column 1, lines 2, 3, 5, and 6). Supernate-treated macrophages, on the other hand, were fully capable of ingesting both E(IgG)C and E(IgM)C-IgG, even after their Fc receptors had been inhibited with immune complexes and aggregates (Table V, columns 2 and 3, and compare with Table IV, columns 2 and 3, lines 1–3).

These results indicate that immune complexes efficiently block the interaction of the macrophage's Fc receptors with particle-bound IgG, even when the particle is coated with C3b as well, and confirm our previous finding (16) that particles coated with both IgG and C3b are phagocytized only via the Fc receptors of normal macrophages. They also demonstrate that the ability of macrophage complement receptors to mediate phagocytosis is critically important to macrophages that have ingested soluble immune complexes, even when the cells encounter a particle coated with both IgG and C3b ligands.

Effect of Ingestion of Complement-treated Soluble Immune Complexes upon Macrophages' Fc and C3 Receptor Function. As previously indicated, coating immobilized immune complexes with complement may mask the Fc portion of the IgG molecule and make it unavailable to macrophage Fc receptors (15). In vivo soluble immune complexes fix complement (14) and are likely to be coated with complement components. Were these components to mask the Fc portion of IgG, then macrophages' Fc receptors could not be engaged by the complexes, the cells could not be triggered to signal lymphocytes to elaborate the lymphokine (1), and their Fc receptors would not be blocked. Moreover, if C3b were on the complexes and available to macrophages' C3b receptors, these receptors might be blocked.

To evaluate the effect of complement treatment of soluble immune complexes upon the ability of complexes to be ingested and to trigger the macrophage function necessary for the generation of supernatant activity, I used complement-treated, soluble HSA-anti-HSA complexes, some of which were rhodamine-labeled, as the material fed to macrophages in the preparation of experimental supernates. Macrophages avidly ingested these complexes, as indicated by fluorescence microscopy, and supernates obtained from cultures in which these macrophages had been co-cultivated with thymocytes were as active as supernates prepared using HSA-anti-HSA complexes that had not been incubated with complement (data not shown). Thus,

previous exposure of soluble immune complexes to complement did not prevent macrophages from ingesting the complexes via their Fc receptors or from acquiring the ability to signal T lymphocytes to elaborate the lymphokine.

Other experiments were designed to determine the effect of exposure of macrophages to complement-treated immune complexes upon the cells' ability to interact with particles via their Fc and C3b receptors. Macrophages were fed either complement-treated HSA-anti-HSA complexes or complement-treated IgG aggregates either after preincubation in supernates (preincubation assay) (Fig. 2, open bars) or before exposure to supernates (direct assay) (Fig. 2, hatched bars). Like their counterparts that had ingested noncomplement-treated complexes and aggregates (Table IV), these macrophages were unable to ingest E(IgG), but ingested E(IgM)C as efficiently as macrophages that had not ingested complexes (Fig. 2).

These results indicate that treating soluble immune complexes with large quantities of complement (50% serum) does not prevent the interaction of the complexes with macrophage Fc receptors or the resulting Fc receptor blockade and that ingestion of these complexes does not block macrophages' C3b receptors or impair the cells' ability to respond to the lymphokine and ingest complement-coated particles.

Discussion

Results of the present experiments indicate that macrophage Fc receptors are functionally (and perhaps physically) blocked when the cell ingests soluble immune complexes; that the phagocytic blockade is selective, for the ability of macrophages to bind and to ingest particles by other means is not impaired; and that the lymphokine that augments the complement receptor function of normal macrophages can also augment the complement receptor function of macrophages whose Fc receptor function has been severely compromised by the ingestion of immune complexes.

In vivo experiments have previously suggested that circulating immune complexes can block the Fc receptors of phagocytic cells. The ability of mice to clear intravenously

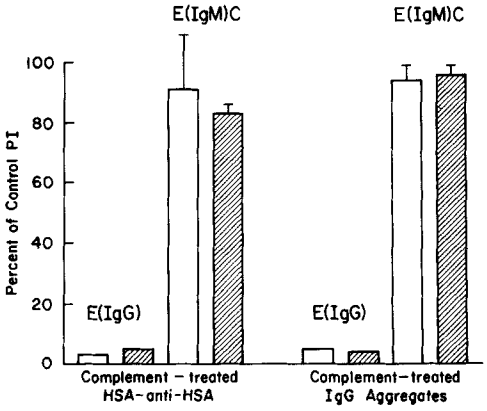

FIG. 2. Effect of ingestion of complement-treated soluble immune complexes upon macrophages' Fc and C3 receptor function. Macrophages were fed either complement-treated HSA-anti-HSA complexes or complement-treated IgG aggregates either after preincubation in supernates (open bars) or before exposure to supernates (hatched bars). The cells' ability to ingest E(IgG) and E(IgM)C was then assessed. Percent of Control PI (ordinate) is the percent of the PI of E(IgG) or E(IgM)C by macrophages that had been treated with supernates but which had not been pretreated with immune complexes or aggregates.

administered HSA-anti-HSA complexes diminishes markedly as the dose of complex administered is increased (17). The clearance of radiolabeled, IgG-coated erythrocytes from the circulation occurs much more slowly in patients with systemic lupus erythematosus than in normal controls, and the degree of impairment is directly related to the level of circulating immune complexes in the patients (18). The ability to ingest IgG-coated particles is likewise diminished in polymorphonuclear leukocytes of patients with rheumatoid arthritis (19). That ingestion of soluble immune complexes in vivo is responsible for this defect is supported by the finding that treatment of rheumatoid patients with penicillamine, a drug that disrupts disulfide bonds in rheumatoid factor (20) and thereby diminishes the level of circulating immune complexes, restores to normal the ability of the their polymorphonuclear leukocytes to ingest IgG-coated latex beads (19).

Isolated IgG and the Fc fragment of IgG can block the binding and uptake of IgG-coated particles by phagocytic cells in vitro (21–27). These findings suggest that soluble immune complexes may also block Fc receptors, and results of the present experiments confirm that they can. Macrophage Fc receptor function can also be blocked by plating the cells on immobilized immune complexes (15, 28–31); here, even the function of receptors on the nonbound surface of the macrophage is impaired. In each of these studies, the Fc receptor blockade was clearly mediated by immobilized and not by soluble complexes. In fact, Rabinovitch et al. (28) were unable to block Fc receptor function with soluble complexes. The reasons for the discrepancy between my findings and theirs are not known but may be technical. For example, I used complexes containing 20 μg or more HSA, whereas they used soluble complexes containing only 4 μg of bovine serum albumin. Furthermore, because the degree of immune complex-mediated Fc receptor blockade observed in my studies was dependent upon the concentration of anti-E IgG used to opsonize the test erythrocytes (Fig. 1), use of higher titers of opsonizing antibody in their experiments might have masked the blockade. Like immobilized immune complexes (15), soluble immune complexes appear to block trypsin-resistant but not trypsin-sensitive immune complexes (Table II).

In each of the studies cited above, blockade of macrophage Fc receptor function by immoblized complexes did not impair the function of other receptors that promote particle binding and ingestion (15, 28–31). Likewise, in the present experiments, ingestion of soluble complexes did not alter macrophages' ability to phagocytize by other means. The mechanism by which complexes selectively block Fc receptor-mediated functions is not known. There are two general possibilities. Immune complexes may bind to, and result in clearance from the plasma membrane of, the vast majority of Fc receptors. Alternatively, binding or clearance of a minority of Fc receptors may trigger events that lead to qualitative or quantitative alterations in the remaining Fc receptors.

There are several mechanisms by which engagement or clearance of a minority of Fc receptors might modify the interaction of the remaining receptors with IgG-coated particles. (*a*) It might alter the structure and/or binding affinity of the remaining receptors. Evidence has recently been presented that suggests that engagement of immobilized immune complexes by Fc receptors located on only a portion of the macrophage plasma membrane inhibits the function of Fc receptors located on the free macrophage surface by an ATP-dependent mechanism (31). One possible expla-

nation for those findings is that a cAMP-mediated reaction, triggered by engagement of some of the cell's Fc receptors, resulted in a structural change in the remaining Fc receptors. (*b*) Engagement or clearance of a minority of Fc receptors might disengage remaining Fc receptors from a transmembrane communication network so that binding of particle-bound IgG to remaining receptors fails to generate the phagocytic signal. Such an effect might be mediated by cAMP or any of a number of other intracellular or plasma membrane components. This hypothesis does not readily explain how the blockade is selective for Fc receptor function and does not seem likely in view of my finding that nearly all trypsin-resistant Fc receptor binding activity was removed by immune complex treatment of macrophages (Table II). (c) Michl et al. (15) have suggested that receptor aggregation may be a prerequisite for phagocytosis. Thus, engagement or clearance of a minority of Fc receptors might freeze other Fc receptors so that they cannot aggregate, or patch, when engaged by particle-bound IgG. Each of the above mechanisms is possible, especially when blockade is mediated by immobilized immune complexes. However, we have previously found that engagement of Fc receptors by particle-bound IgG on one portion of the macrophage plasma membrane does not interfere with the binding or phagocytic function of Fc receptors located on other portions of the cell's surface (16). Thus, if the interaction of macrophage Fc receptors with soluble immune complexes is similar to their interaction with IgG-coated particles, then none of the above mechanisms seems a likely explanation for the blockade of Fc receptor function by soluble complexes.

The simplest interpretation of my results is that ingestion of immune complexes results in clearance of most phagocytosis-competent Fc receptors from the macrophage surface, leaving too few available to bind and to promote the ingestion of IgG-coated particles. Internalization of most of the macrophage's Fc receptors would probably result in internalization of a large percentage of the cell's plasma membrane, raising the question of how complement receptors and other particle-binding sites could avoid being passively interiorized as well. Tsan and Berlin (32) have shown that some plasma membrane components are selectively excluded from a phagocytic vacuole. Therefore, my finding that the phagocytic blockade induced by immune complexes is selective for Fc receptors is not inconsistent with this simple interpretation.

Had treatment of immune complexes with complement rendered the complexes either incapable of binding to macrophages' Fc receptors or capable of blocking the cells' C3b receptors, then the pathophysiologic relevance of the other findings presented would have been open to serious question. However, treatment of immune complexes with concentrations of complement that approximate those likely to be available at inflammatory sites in vivo did not impair their ability to be recognized by macrophage Fc receptors, and uptake of these complexes by macrophages did not impair the cells' complement receptor function (Fig. 2). Therefore, the fact that complement components are likely to be present on soluble immune complexes in vivo does not appear to diminish the likelihood that selective blockade of macrophage Fc receptor function might occur, and might be important, in a variety of disease states.

Soluble immune complexes are present at most inflammatory sites. My results suggest that macrophages that ingest these complexes are rendered unable to phagocytize via their Fc receptors. Macrophage complement receptors are not normally phagocytic receptors (4, 6, 33). Therefore, at inflammatory sites, macrophages in their

normal physiologic state may be unable to ingest particles coated with IgG, with C3b, or with both ligands. Classical delayed hypersensitivity mechanisms would not enhance these macrophages' ability to ingest opsonized particles, for lymphokines generated by these mechanisms do not result in augmented macrophage complement receptor function (1).

However, we have defined a series of cellular and molecular events whereby macrophages may acquire the ability to phagocytize C3b-coated particles (1). The first step in this series is ingestion of immune complexes by macrophages. Thus, uptake of immune complexes, whereas it paralyzes macrophages' Fc receptor function, at the same time initiates a sequence of cellular interactions that subsequently lead to the elaboration of a lymphokine that imparts to macrophages the ability to phagocytize via their complement receptors. These macrophages may then circumvent the immune complex-induced phagocytic blockade and ingest immunologically coated particles via their C3b receptors.

Summary

The effects of ingestion of soluble immune complexes upon macrophage phagocytic function was studied. Ingestion of immune complexes severely impaired the macrophage's ability to ingest IgG-coated particles but did not alter its ability to interact with particles by means other than its Fc receptors. Treatment of macrophages that had ingested immune complexes with supernates containing the previously described lymphokine that augments macrophage complement receptor function failed to enhance the cells' interaction with either IgG-coated erythrocytes or zymosan particles but markedly enhanced their ability to phagocytize via their complement receptors. The possible significance of these findings in immunologically mediated inflammation is discussed.

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