EFFECTS OF IMMOBILIZED IMMUNE COMPLEXES ON Fc- AND COMPLEMENT-RECEPTOR FUNCTION IN RESIDENT AND THIOGLYCOLLATE-ELICITED MOUSE PERITONEAL MACROPHAGES*

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Soluble or particle-bound immune complexes containing the Fc domains of certain antibody subclasses, and cleavage products of the third component of complement interact with highly specific Fc and complement receptors on the surfaces of cells of the immune system. When these interactions occur on the surfaces of phagocytic leukocytes, they stimulate the selective release of lysosomal enzymes (1, 2), the expression of cell-mediated cytotoxic effects (3, 4), and the attachment and ingestion of particulate materials (5, 6, 7).

During phagocytosis of antibody- or complement-coated particles, the advancing plasma membrane of the phagocyte is guided by the interaction of its receptors with ligands on the surface of the particle (8, 9). The advancing membrane pseudopods and the resulting phagocytic vacuole conform precisely to the geometry of the particle's surface. These findings suggest that there is an excellent correspondence between the distribution of receptors on the membrane of the phagocyte and ligands on the surface of the particle. Particles with rigid cell walls such as bacteria or yeast, provide an immobile framework for antibody or complement molecules bound to them. The efficient interaction of membrane receptors with such immobilized ligands requires that the receptors have considerable lateral mobility within the plane of the phagocyte's membrane.

By using multivalent ligands such as antibodies or lectins, the movement of cell-surface molecules into caps has been demonstrated (10–13). Similarly, the attachment of antibody- or complement-coated particles to lymphocytes and monocytes results in the redistribution of the corresponding receptor sites (14, 15). Although the mechanism(s) that govern the movements of externally disposed membrane proteins are incompletely understood, there is evidence that the lateral movement of these membrane proteins is regulated by the contractile proteins of the cell (16, 17, 18). In addition, transmembrane associations of surface proteins with cytoskeletal elements are probably important for cell motility and cell spreading, and the remodeling of the plasma membrane during these activities (19, 20).

The spreading of a phagocyte on ligand-coated surfaces is analogous in many respects to the ingestion of ligand-coated particles (21, 22). The surface may be

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considered a giant particle for this purpose. Geometrically fixed ligands such as antibody-antigen complexes or complement on a culture-dish surface should induce cellular responses similar to those occurring during ingestion of particles coated with the same ligands. Moreover, the ligand-coated surface provides an opportunity to functionally polarize the cell's membrane into two domains: a surface-adherent domain within which specific receptors may be focused and engaged by the corresponding ligands on the dish surface, and a nonadherent domain whose composition may be altered as a result of ligand-receptor interaction in the adherent domain. By studying the properties of the nonadherent portion of the cell surface, it should be possible to analyze the effects of surface-adherent ligands on receptor localization and mobility. That such effects occur was first reported by Rabinovitch et al. (23) who showed that macrophages plated on antibody-antigen complexes lose the capacity to ingest, but not to bind, IgG-coated erythrocytes. The presence of complement in the substrate adherent immune complexes, however, did not appear to alter the capacity of macrophages to bind complement-coated erythrocytes (24, 25).

We have developed a simple technique for homogeneously coating glass coverslips and plastic Petri dishes with a variety of immune complexes containing antibodies, or antibodies and complement. Macrophages plated on these surfaces exhibit ligand-specific modulation of their membrane receptors for immunoglobulins and for complement. Our results indicate that surfaces coated with these ligands are powerful tools for dissecting the mechanisms that control receptor movement, and the configuration of the ligands that regulate these activities. They further show that the membranes of macrophages plated onto such ligand-coated surfaces are functionally polarized into substrate-adherent and nonadherent domains.

Materials and Methods

Macrophages. Peritoneal macrophages were obtained as described (26) from NCS mice (Laboratory Animal Research Center, The Rockefeller University, New York) either by injecting phosphate-buffered saline deficient in Ca^{++} and Mg^{++} ions (PD)[1] (27) intraperitoneally and collecting the cells (PC) immediately thereafter (resident macrophages), or by injecting 2 ml brewer's thioglycollate (Difco Laboratories, Detroit, Mich.) intraperitoneally and collecting the peritoneal exudate cells (PEC) after 4–5 d (thioglycollate-elicited macrophages). 4×10^5 PC or 2×10^5 PEC were seeded into each 16-mm well of Costar plates (Costar, Data Packaging, Cambridge, Mass.). Each well contained a glass coverslip treated as described below, and 0.5 ml Eagle's minimum essential medium with Earle's salt solution (MEM) (Grand Island Biological Co., Grand Island, N. Y.). The Costar plates were maintained for 30–60 min at 37°C in a 5% CO_2-95% air mixture. The coverslips were washed free of nonadherent peritoneal cells, and transferred into 0.5 ml MEM in fresh Costar plates.

Trypsinization of Peritoneal Cells. Peritoneal cells (4×10^6 cells/ml) in warm phosphate-buffered saline with Ca^{++} and Mg^{++} ions (PBS) (27), and containing 0.75 mg/ml trypsin (Worthington Biochemical Corp., Freehold, N. J.) and 0.025 mg/ml DNAase (Sigma Chemical Co., St. Louis, Mo.) were incubated in a plastic tube (Falcon No. 2057; Falcon Labware, Div. of Becton, Dickinson & Co., Oxnard, Calif.) at 37°C for 20 min on a rotating wheel (20 rpm).

[1] *Abbreviations used in this paper:* BSA, bovine serum albumin; DNP, dinitrobenzene; E, sheep erythrocytes; E(IgG), E coated with rabbit anti-E IgG; E(IgG2a), E coated with monoclonal mouse anti-E IgG2a; E(IgM)C, E coated with rabbit anti-E IgM and mouse complement; hFBS, heat-inactivated fetal bovine serum; HRP, horseradish peroxidase; HRP-GαR IgG, HRP-labeled goat IgG against rabbit IgG; MEM, Eagle's minimum essential medium with Earle's salt solution; PBS, phosphate-buffered saline with Ca^{++} and Mg^{++} ions; PC, resident mouse peritoneal cells; PD, PBS deficient in Ca^{++} and Mg^{++} ions; PEC, mouse peritoneal exudate cells; PLL, poly-L-lysine; RαBSA IgG, rabbit anti-BSA IgG; RαDNP $F(ab')_2$, $F(ab')_2$ fragments of RαDNP IgG; RαDNP IgG, rabbit anti-DNP IgG; RαHRP IgG, rabbit anti HRP IgG; VBG, Veronal-buffered glucose with divalent cations

The digestion process was stopped with an equal volume of cold PD and 10% heat-inactivated (30 min, 56°C) fetal bovine serum (hFBS) (Flow Laboratories, Inc., Rockville, Md.). The cells were washed once with PD and 10% hFBS, and once in PD alone, all at 2.5°C, and resuspended in cold MEM at a concentration of 4×10^6 PC/ml or 2×10^6 PEC/ml. Cells were >95 viable as examined by trypan blue (Grand Island Biological Co.) (28) exclusion after each trypsinization. The average cell recovery of trypsinized cells was 60%. Cells exposed to the same treatments in absence of trypsin showed an average cell recovery of 82% with >95% of the cells viable.

Preparation of Coverslips. Glass coverslips of 12-mm Diam (SGA Scientific, Inc., Bloomfield, N. J.) were treated overnight at room temperature with chromerge (Manostat Corp., New York) dissolved in concentrated sulfuric acid, and washed for 2–4 h in running water followed by 1 h in distilled water. At this point, the coverslips were either dried, and stored as ready for use, or dried, and incubated in PD containing 0.1 mg/ml poly-L-lysine (PLL) (mol wt, ≅70,000; Miles Laboratories Inc., Miles Research Products, Elkhart, Ind.) for 30 min at room temperature. The PLL-coated coverslips were washed with PD, drained, and reacted with 16.8 mg/ml 2,4-dinitrobenzene (DNP) sulfonate (Eastman Kodak Co., Rochester, N. Y.) in 0.15 M sodium-carbonate buffer (pH 11.6) for 30 min at room temperature. To bind bovine serum albumin (BSA) (Sigma Chemical Co.) or horseradish peroxidase (HRP) (Sigma Chemical Co.) the PLL-coated coverslips were first treated with 2.5% glutaraldehyde in PD for 15 min at room temperature, washed extensively in distilled water followed by PD, and then reacted with 1 mg/ml BSA or 10 mg/ml HRP in PD for 30 min at room temperature. To quench unreacted aldehyde groups, the coverslips were treated overnight at room temperature with 0.2 M glycine in 0.01 M sodium-phosphate buffer (pH 7.2). Samples of coverslip preparations that were assayed for bound HRP as described (29), demonstrated from 55 to 89 ng HRP linked to the surface of the coverslips. More than 90% of macrophages cultured in MEM on these coverslips for up to 4 h at 37°C in absence of serum excluded trypan blue (28).

Substrate-adherent antibody-antigen complexes were formed by incubating PLL-DNP-coated coverslips with 9–13 μg of rabbit anti-DNP IgG (RαDNP IgG) in 60 μl PBS for 30 min at room temperature. The coverslips were then washed with PD and used on the same day. In experiments to be reported elsewhere (J. Michl, M. M. Pieczonka, J. C. Unkeless, and S. C. Silverstein. Manuscript in preparation.), we found that this amount of RαDNP IgG had a maximal effect on Fc-receptor function. Untreated coverslips, and coverslips incubated with rabbit anti-BSA IgG (RαBSA IgG), RαDNP IgG, or with DNP alone served as controls. RαDNP IgG was produced in rabbits immunized with DNP-keyhole limpet hemocyanin in complete Freund's adjuvant, and was purified by affinity chromatography by the method of Eisen et al. (30), modified as described (31). $F(ab')_2$ fragments of this antibody (RαDNP $F[ab']_2$) were obtained by pepsin digestion of RαDNP IgG as described (32). RαBSA IgG was purified by DEAE-cellulose chromatography (33), and used at a concentration of 10–16 μg per coverslip. Rabbit anti-HRP IgG (RαHRP IgG) was a gift of Dr. M. L. Yarmush, The Rockefeller University. Immobilized BSA-RαBSA IgG and HRP-RαHRP IgG complexes, respectively, were prepared following the procedure described above for the formation of substrate-adherent DNP-RαDNP IgG complexes.

Complement-coated coverslips were prepared by incubating the PPL-DNP-RαDNP IgG-coated coverslips for 10 min at 37°C with 0.075 ml per coverslip of NCS mouse serum diluted 1:1 in Veronal-buffered glucose (VBG) with divalent cations (34). The coverslips were then rinsed with PD (2.5°C) and used immediately for the seeding of peritoneal cells. In control preparations heat-inactivated (30 min, 56°C) mouse serum was used instead of fresh mouse serum.

Preparation of Particles. Sheep erythrocytes (E) (Laboratory Animal Research Center, The Rockefeller University) were coated with either rabbit anti-E IgG (Lot 50767, Cordis Laboratories, Inc., Miami, Fla.) (E[IgG]) or with rabbit anti-E IgM (Lot 90906, Cordis Laboratories, Inc.) (E[IgM]) as described (7). The rabbit IgM was absorbed with *Staphylococcus aureus* to remove residual IgG (35). To prepare complement-coated E (E[IgM]C) the E(IgM) were further incubated with freshly thawed mouse serum (stored at −75°C) diluted 1:5 in VBG is described (7). Monoclonal mouse anti-E IgG2a, which was used to make E opsonized with a subagglutinating dose of monoclonal mouse anti-E IgG2a (E[IgG2a]), was prepared and kindly given to us by Diamond et al. (The Albert Einstein College of Medicine, Yeshiva University, New York)

(36). E(IgG), E(IgG2a), and E(IgM)C were adjusted to a final concentration of 2.5×10^8E/ml PD by measurement of the hemoglobin concentration and the use of a standard curve.

Binding and Phagocytosis Assay. Macrophages on coverslips were placed into 0.5 ml MEM in Costar wells. 0.1 ml of E(IgG) or E(IgM)C was added to each well and the complexes were incubated for 60 min at 37°C. The coverslips were then removed from the wells, dipped into PD to remove nonattached E from the macrophages, or treated with hypotonic buffer to lyse E that were attached but not ingested, and fixed in 1.25% glutaraldehyde in PD. The number of E attached or ingested was determined by phase-contrast microscopy. At least 100 macrophages in random fields were counted. The percentage of macrophages that attached or ingested E multiplied by the average number of E attached or ingested per macrophage is expressed as attachment and ingestion index, respectively.

Electron Microscopy. 35-mm tissue-culture dishes (Falcon No. 3001; Falcon Labware, Div. of Becton, Dickinson & Co.) were coated with PLL-DNP as described above for cover slips omitting the chromerge-sulfuric acid treatment, and then incubated with 1 ml of PBS containing 0.28 mg of RαDNP IgG. To visualize the surface-adherent immune complexes these dishes were further incubated for 30 min at 2.5°C with HRP-labeled goat IgG against rabbit IgG (HRP-GαR IgG) (lot No. 11059, N. L. Cappel Laboratories Inc., Cochranville, Pa.). The Petri dishes were washed by repeated dipping into cold PD. For controls, dishes coated with PLL alone or with PLL and DNP were overlaid with HRP-GαR IgG or the HRP-GαR IgG treatment was omitted. Staining for HRP was performed according to the method of Graham and Karnovsky (37) using diaminobenzidine (Sigma Chemical Co.) as substrate.

For electron microscopy, 4×10^6 PEC or 6–8 $\times 10^6$ PC were plated for 30 min at 37°C. The samples were processed as described (38), and the sections examined in a Philips 201 electron microscope (Philips Electronic Instruments, Inc., Mahwah, N. J.). In some preparations the cells were embedded *in situ* and sectioned perpendicularly to the dish surface to provide optimal views of the contact zone between macrophages and the immune-complex substrate, and of the macrophage surfaces not adherent to the dish.

In parallel preparations, 2×10^6 PEC or 6×10^6 PC in MEM were cultured on control or immune complex-coated dishes, and the effect on attachment and ingestion of E(IgG) was examined. It was found that the results resembled the findings on the glass-coverslip preparations.

Results and Discussion

Effects of Immobilized Antibody-Antigen Complexes on Fc-Receptor Function. Thioglycollate-elicited macrophages plated on coverslips coated with DNP-RαDNP IgG complexes exhibited a 68% reduction in attachment and a 97% reduction in ingestion of E(IgG). No inhibition of attachment or ingestion of E(IgG) was seen when macrophages were cultured on coverslips coated with PLL-DNP alone, or with PLL-DNP and RαDNP $F(ab')_2$ (Table I). No significant alteration in Fc-receptor function was noted when macrophages were cultured on PLL-coated coverslips that had been incubated with RαDNP IgG, or on PLL-DNP-coated coverslips that had been incubated with RαBSA IgG. Qualitatively similar results were obtained when resident macrophages were substituted for thioglycollate-elicited macrophages in a parallel series of experiments.

These results confirm the requirement for the Fc portion of antigen-specific IgG in the substrate-adherent immune complexes to induce paralysis of Fc-receptor-mediated phagocytosis (23). In addition, the decreased capacity of macrophages plated on antibody-antigen complex-coated surfaces to bind E(IgG) suggests that Fc receptors have been removed from, or inactivated on, the portion of the macrophage membrane not in contact with the substrate.

Localization of Antibody-Antigen Complexes. One source of error in experiments with

TABLE I

Effect of Immobilized Antibody-Antigen Complexes on Fc- and Complement-Receptor-mediated Phagocytosis by Thioglycollate-elicited Mouse Macrophages

Treatment of coverslips coated with PLL-DNP	E(IgG)				E(IgM)C			
	Percentage of macrophages attaching	Attachment index	Percentage of macrophages ingesting	Ingestion index	Percentage of macrophages attaching	Attachment index	Percentage of macrophages ingesting	Ingestion index
	%		%		%		%	
None	62	930	90	1,332	98	1,470	64	653
RαDNP IgG	31	295	7	32	99	1,980	60	342
RαDNP $F(ab')_2$	90	744	93	791	ND		ND	
RαBSA IgG	82	1,054	85	638	ND		ND	

Preparation of coverslips, culture conditions, binding, and phagocytosis assays as described in Materials and Methods.
ND, not done.

immobilized ligands is that the effects observed may be due to the removal of the ligand from the substrate. Proteases secreted by macrophages might release immune complexes from the coverslips into the medium. These soluble immune complexes might bind to and block the Fc receptor on the nonadherent membrane surface. Moreover, the immune complexes that bind initially to membrane receptors on the substrate-adherent surface might move in the plane of the membrane to the nonadherent surface of the macrophage. Using a similar system, Rabinovitch et al. (23) demonstrated that immune complexes were not released into the medium, and that soluble immune complexes added to the culture vessel were not responsible for the inhibition observed. However, they did not examine the possibility of movement of immune complexes in the plane of the membrane. To resolve these issues unambiguously we treated DNP-RαDNP IgG-coated Petri dishes with HRP-GαR IgG. After vigorous washing to remove loosely adsorbed IgG from the surfaces of the culture dishes, macrophages were plated on them, and incubated for 30 min at 37°C. As shown in the electron micrographs in Fig. 1, HRP-GαR IgG was found exclusively on the substrate-adherent cell surface. No peroxidase label was observed on the upper surface of the macrophages; in a few sections peroxidase label was seen in the macrophage cytoplasm in vacuoles directly adjacent to the substrate-adherent plasma membrane. These experiments demonstrate that the immune complexes are not released from the substrate, that they are not present on the upper surface of the macrophage, and that they are not moved to other cellular compartments during the course of these experiments. Thus, loss of Fc-receptor activity in macrophages plated on DNP-RαDNP IgG-coated surfaces is not a result of Fc-receptor blockade by immune complexes bound to the portion of the macrophage membrane that is not in contact with the substrate.

Residual Fc-Receptor Activity. Mouse peritoneal macrophages express two Fc receptors: a protease-resistant Fc receptor that mediates binding and ingestion of particles coated with aggregated rabbit IgG or aggregated mouse IgG of subclasses 1 and 2b,

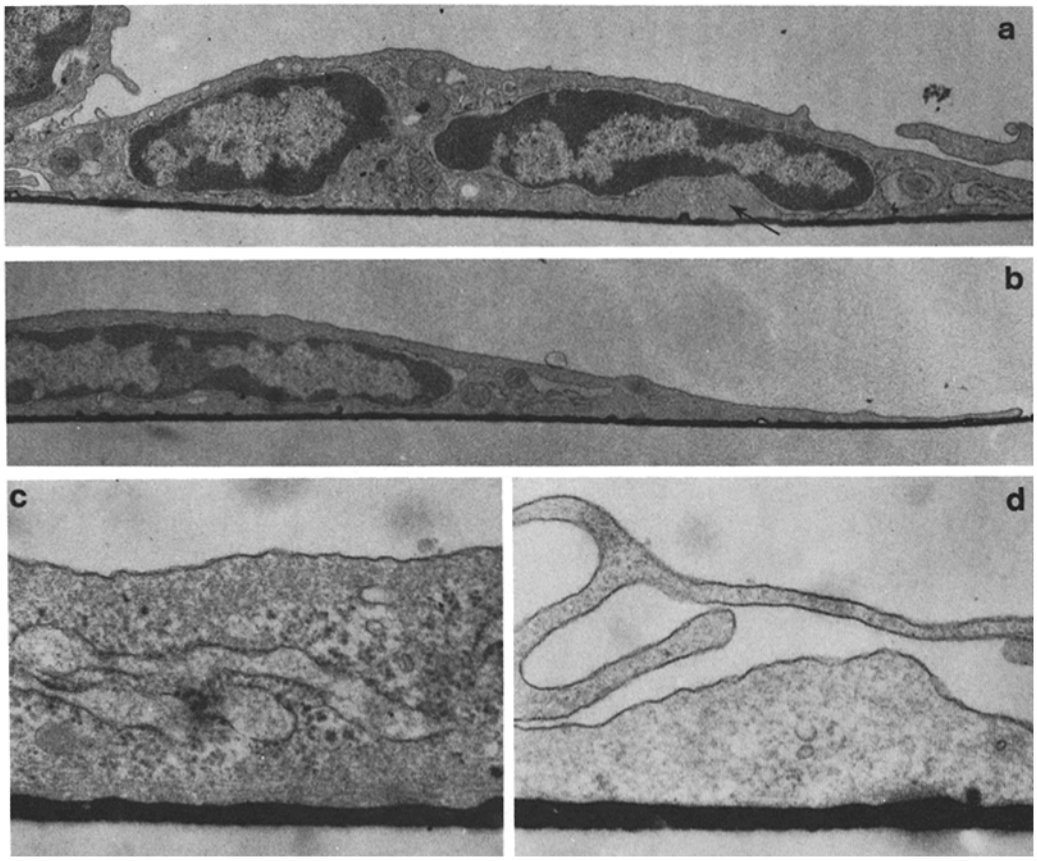

FIG. 1. Electron micrographs of unstained sections of resident mouse peritoneal macrophages cultured for 30 min at 37°C on DNP-RαDNP IgG and HRP-GαR IgG complexes. The macrophages adhere tightly to the peroxidase-labeled and diaminobenzidine-stained antibody-antigen complexes on the dish surface. No peroxidase label can be found on the nonadherent surface of the cell body (a, b, and c) or on the extending pseudopods (b and d). Note the absence of stimulation of pinosome formation on the side of contact between macrophages and dish-adherent antibody-antigen complexes, and the presence of zones of organelle exclusion (a, arrow) which contain microfilaments running mostly parallel to the dish-surface-adherent plasma membrane (c). a and b, × 4,900; c, × 27,000; d, × 32,400.

and a trypsin-sensitive Fc receptor that binds monomeric and aggregated mouse IgG of subclass 2a (IgG2a receptor) (36, 39, 40). Particles coated with rabbit IgG bind to both types of receptors (36, 39). Because in our experiments rabbit IgG was bound to antigens on the dish surface (Table I) and to the E used to assay for receptor activity, it was important to determine which of these receptors was responsible for the residual binding of E(IgG) by macrophages plated onto DNP-RαDNP IgG complexes.

To answer this question, and to examine the behavior of the protease resistant Fc receptors in the absence of the IgG2a receptor, macrophages were incubated with trypsin before plating (Fig. 2). Whereas trypsinization failed to affect the attachment of E(IgG) to macrophages on control coverslips, it reduced by >95% the binding of E(IgG) to macrophages plated onto DNP-RαDNP IgG-coated coverslips (Table II). These results indicate that trypsin-sensitive Fc receptors remain on the nonadherent

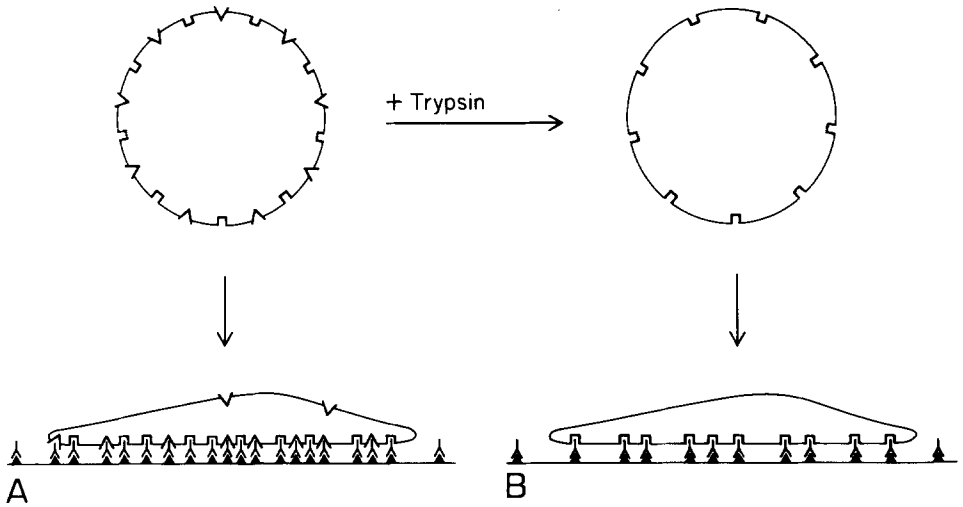

FIG. 2. Model of the effect of trypsin on the Fc-receptor activity of macrophages plated onto rabbit IgG-antigen complexes. For further description see text. Symbols (⌐¬), trypsin-resistant Fc receptor; (⌐V¬) trypsin-sensitive Fc receptor; (_▲_) surface-adherent antigen; (⅄) rabbit IgG directed against the surface-adherent antigen.

surface of macrophages plated onto rabbit antibody-antigen complexes; they suggest that these receptors are responsible for the residual binding of E(IgG). When these Fc receptors are removed by trypsin treatment, no Fc receptors are left on the nonadherent surface of the phagocytes.

To examine directly the presence or absence of the trypsin-sensitive Fc receptor that binds mouse IgG2a we used E(IgG2a). As shown in Table II, macrophages plated on control coverslips bound and ingested 8–10 E(IgG2a) each. In contrast most of the macrophages trypsinized in suspension before plating on control coverslips failed to bind or ingest any E(IgG2a), confirming that E(IgG2a) are selectively bound by the macrophages' trypsin-sensitive Fc receptors (36).

Macrophages plated on DNP-RαDNP IgG complexes continued to bind and ingest E(IgG2a), albeit in markedly reduced numbers. However macrophages that had been trypsinized before plating on similarly coated coverslips neither bound nor ingested E(IgG2a). We propose the following model (Fig. 2) and explanation for these results: the presence of DNP-RαDNP IgG complexes on the surface of the coverslips induces the complete removal of trypsin-resistant Fc receptors from the nonadherent part of the macrophage plasma membrane (Fig. 2B). These receptors mediate the phagocytosis of E coated with rabbit IgG (E[IgG]), and their removal results in the virtually complete inhibition of ingestion of these particles. The trypsin-sensitive Fc receptors for mouse IgG2a are incompletely modulated by the RαDNP IgG in the substrate-adherent immune complexes (Fig. 2A). Although we cannot explain the lack of complete modulation of IgG2a receptors by the substrate-adherent rabbit antibody-antigen complexes, it is evident that IgG2a receptors are responsible for the residual binding of E(IgG) and the residual ingestion of E(IgG2a) by macrophages plated on rabbit antibody-antigen complexes.

Complement-Receptor Modulation. To determine whether membrane receptors other than the Fc receptors are modulated when macrophages are plated on ligand-coated

TABLE II

Effect of Trypsin on the Attachment and Ingestion of E(IgG) and E(IgG2a) by Thioglycollate-elicited Mouse Macrophages Cultured on Immobilized DNP-RαDNP IgG Complexes

Treatment of coverslips coated with PLL-DNP	Treatment of macrophages	E(IgG2a)				E(IgG)			
		Percentage of macrophages attaching	Attachment index	Percentage of macrophages ingesting	Ingestion index	Percentage of macrophages attaching	Attachment index	Percentage of macrophages ingesting	Ingestion index
		%		%		%		%	
None	—	86	602	76	707	62	930	90	1,332
RαDNP IgG	—	32	173	26	135	31	295	7	32
None	Trypsin	14	70	19	72	74	518	84	924
RαDNP IgG	Trypsin	3	14	0	0	4	24	2	25

E(IgG2a) were adjusted to 2.5×10^8 E/ml in PD, and 0.1 ml of this suspension was added to each Costar well. Trypsinization of macrophages, binding, and phagocytosis assays were performed as described in Materials and Methods.

surfaces, we examined the effects of substrate-bound complement on complement-receptor activity of thioglycollate-elicited macrophages. Coverslips coated with DNP-RαDNP IgG were incubated with freshly thawed mouse serum as a complement source. Under these conditions, the immune complexes promote complement fixation to the surface of the coverslip. As expected (41), resident and thioglycollate-elicited macrophages rapidly spread out on these immune complex- and complement-coated coverslips. E(IgM)C were then added to these macrophages. As shown in Table III, line 2 and Table IV, line 3, there was a marked reduction in binding and ingestion of E(IgM)C when thioglycollate-elicited macrophages were plated on complement-coated coverslips. No decrease in complement-receptor activity was observed when thioglycollate-elicited marcophages were plated on DNP-RαDNP IgG-coated coverslips that had been incubated with heat-inactivated mouse serum (Table III, line 5), or when DNP-coated coverslips were treated with RαBSA IgG or RαHRP IgG and fresh mouse serum (Table III, lines 5 and 6). Complement-receptor modulation was observed when thioglycollate-elicited macrophages were plated on coverslips treated with DNP, RαDNP $F(ab')_2$ and fresh mouse serum. $F(ab')_2$ antibody fragments promote complement fixation via the alternate pathway (42). Thus, surface-adherent antibody-antigen complexes and an active complement source are required to promote complement-receptor modulation in thioglycollate-elicited macrophages. Scharfstein et al., (43) have shown that antibody-antigen complexes bind complement components C3 and C4, but do not bind the terminal complement components C5–9. Because in the presence of fresh serum, both the activator, DNP-RαDNP IgG, of the classical complement pathway and the activator, DNP-RαDNP $F(ab')_2$, of the alternative complement pathway promote complement-receptor modulation, we suggest that C3b bound to immune complexes on the substrate is responsible for this effect.

Complement Receptors of Resident Macrophages Do Not Modulate. Both resident and thioglycollate-elicited mouse peritoneal macrophages possess a trypsin-sensitive membrane receptor for the third component of complement, C3b. The complement receptor of thioglycollate-elicited macrophages promotes both attachment and ingestion of E(IgM)C. The complement receptor of resident macrophages mediates only the attachment of E(IgM)C (44). These differences in function of the complement receptors of resident and thioglycollate-elicited macrophages led us to search for

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TABLE III

Effect of Different Coverslip Preparations on the Modulation of the Complement Receptor of Thioglycollate-elicited Mouse Macrophages

Treatment of coverslips coated with PLL-DNP	Percentage of macrophages with E(IgM)C attached	Average number of E(IgM)C attached per macrophage	Attachment index
	%		
RαDNP IgG	84	8	672
RαDNP IgG + mouse serum (C3b)	26	6.5	169
RαDNP $F(ab')_2$ + mouse serum (C3b)	33	8	264
Mouse serum	72	10	720
RαBSA IgG + mouse serum	82	9.5	779
RαHRP IgG + mouse serum	78	9	702
RαDNP IgG + mouse serum (30 min, 56°C)	83	8	664

Preparation of coverslips as described in Materials and Methods. The binding assay was performed with 0.05 ml E(IgM)C added to each Costar well (concentration 2.5×10^8 E/ml in PD) for 1 h at 37°C.

TABLE IV

Effect of Complement-containing DNP-RαDNP IgG Complexes on Complement-Receptor Activity in Resident and Thioglycollate-elicited Mouse Macrophages

Treatment of coverslips coated with PLL-DNP	Resident macrophages		Thioglycollate-elicited macrophages			
	Percentage of macrophages with E(IgM)C attached	Attachment index	Percentage of macrophages with E(IgM)C attached	Attachment index	Percentage of macrophages with E(IgM)C ingested	Ingestion index
	%		%		%	
None	85	1,105	91	1,365	84	798
RαDNP IgG	94	705	96	1,055	80	928
RαDNP IgG + mouse serum (C3b)	84	1,176	30	222	31	133

Assay conditions as described in Materials and Methods.

differences in receptor modulation when these macrophages were cultured on complement-coated surfaces. Resident or thioglycollate-elicited macrophages were plated onto complement-coated coverslips and then incubated with E(IgM)C. The complement-receptor activity of thioglycollate-elicited macrophages was markedly reduced (Table IV). In contrast, there was no reduction in the attachment of E(IgM)C by resident macrophages. As expected, ingestion of E(IgM)C by resident macrophages was not observed. The presence of DNP or DNP-RαDNP IgG complexes on the coverslips had no significant effect on the attachment and ingestion of E(IgM)C by thioglycollate-elicited macrophages, or on the binding of E(IgM)C by resident macrophages (Table IV).

These findings suggest that there is a close correlation between the capacity of macrophages to modulate their membrane receptors and the ability of the receptors

TABLE V

Fc-Receptor-mediated Phagocytosis in Thioglycollate-elicited Mouse Macrophages Cultured on Complement-containing DNP-RαDNP IgG Complexes

Treatment of coverslips coated with PLL-DNP	Percentage of macrophages with E(IgG) attached	Attachment index	Percentage of macrophages with E(IgG) ingested	Ingestion index
	%		%	
None	81	705	91	1,411
RαDNP IgG	51	510	19	152
RαDNP IgG + mouse serum (C3b)	92	1,215	98	1,353
RαDNP IgG + mouse serum (30 min, 56°C)	50	530	12	52

Assay conditions as described in Materials and Methods.

to mediate phagocytosis. Cells whose Fc receptors are modulated upon presentation of immobilized antibody-antigen complexes (resident and thioglycollate-elicited macrophages), or whose complement receptors are modulated upon presentation of complement-coated immune complexes (thioglycollate-elicited macrophages) phagocytose particles coated with the corresponding ligand. However, resident macrophages do not modulate their complement receptors in response to complement in the immobilized complexes and do not phagocytose E(IgM)C.

Specificity of Receptor Modulation. To determine whether the effects of plating macrophages on one type of immobilized ligand affected binding of test particles coated with a different ligand, we examined complement-receptor function in thioglycollate-elicited macrophages plated on DNP-RαDNP IgG complexes. As shown in Table I, no reduction in the capacity of these macrophages to bind E(IgM)C was observed, and the proportion of macrophages that phagocytozed E(IgM)C was undiminished. The average number of E(IgM)C ingested by each macrophage, however, was decreased. Conversely, Fc-receptor function was unaffected when thioglycollate-elicited macrophages were plated on DNP-RαDNP IgG-and-complement-coated surfaces; >90% of these macrophages bound and ingested 13–14 E(IgG) each (Table V, line 3). Thioglycollate-elicited macrophages plated on DNP-RαDNP IgG-coated coverslips that were not further treated, or that were treated with heated mouse serum showed marked reduction in binding, and an almost total inhibition of ingestion of E(IgG) (Table V, lines 2 and 4). These results indicate that substrate-adherent immune complexes affect only those receptors for which they act as ligands. Furthermore, they show that immobilized immune ligands do not cause a general paralysis of macrophage-receptor function.

Although we do not know the mechanism(s) by which these receptors are modulated, it is important to point out that the receptors must move from the upper surface of the macrophage membrane before their interaction with ligands on the substrate. A similar situation has been described for the movement of surface immunoglobulins on motile lymphocytes (45). The capacity of these cells to modulate receptors that have not been complexed with ligands distinguishes these phenomena from ligand-induced lymphocyte capping (46), or the movement of concanavalin A binding sites

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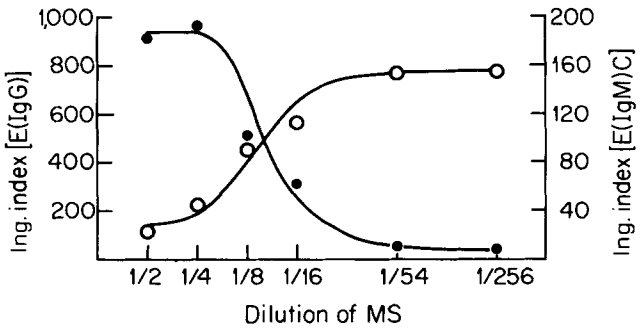

FIG. 3. Effect of the concentration of mouse serum used in the preparation of DNP-RαDNP IgG-and-complement-coated coverslips on Fc- and complement-receptor-mediated phagocytosis by thioglycollate-elicited macrophages. Parallel cultures of macrophages on the different coverslip preparations were incubated for 30 min at 37°C before being exposed to E(IgG) or E(IgM)C, respectively, as described in the text. (○), ingestion (Ing.) index, E[(IgM)C]; (●) ingestion index, [E(IgG)].

in dividing macrophage cell lines (47). In these latter systems, receptors are modulated only when they are complexed by specific ligands.

We were surprised to find Fc-receptor function unimpaired when macrophages were plated on DNP-RαDNP IgG-coated coverslips that had been treated with fresh serum. To be certain that this reflected a general effect of serum on antigen-antibody complexes, we prepared coverslips coated with BSA-RαBSA IgG or with HRP-RαHRP IgG complexes, and incubated them with fresh mouse serum. Similar results were obtained. Thioglycollate-elicited macrophages plated on these coverslips exhibited a marked reduction in complement-receptor activity as measured by E(IgM)C ingestion, but retained full Fc-receptor function as measured by phagocytosis of E(IgG) (data not shown). Thus, the failure of DNP-RαDNP IgG complexes treated with mouse serum to promote Fc-receptor modulation is not a unique property of this IgG preparation. The inability of heated mouse serum to block Fc-receptor modulation by these complexes (Table V, line 4) indicates that complement fixation is required to prevent the interaction of the Fc segments of substrate-adherent antibody-antigen complexes with macrophage Fc receptors.

These results suggested that a reciprocal relationship might exist between the amount of complement fixed by substrate-adherent DNP-RαDNP IgG complexes and the capacity of these complexes to modulate macrophage Fc receptors. To examine this possibility, thioglycollate-elicited macrophages were plated on DNP-RαDNP IgG-coated coverslips that had been incubated with decreasing concentrations of fresh mouse serum. These macrophages were then tested for their ability to ingest E(IgG) or E(IgM)C. As shown in Fig. 3, coverslips incubated with high concentrations of serum inhibited complement-receptor function and had no effect on Fc-receptor activity. At lower serum concentrations, complement-receptor function was unimpaired while Fc-receptor activity was inhibited. The reciprocal relationship between the activities of the two receptors at intermediate serum concentrations was particularly striking. These findings support the concept of an inverse relationship between the amount of complement bound to the substrate-adherent antibody-antigen complexes and the capacity of these complexes to modulate Fc- and complement-receptor function, and are in agreement with the model proposed in Fig. 4.

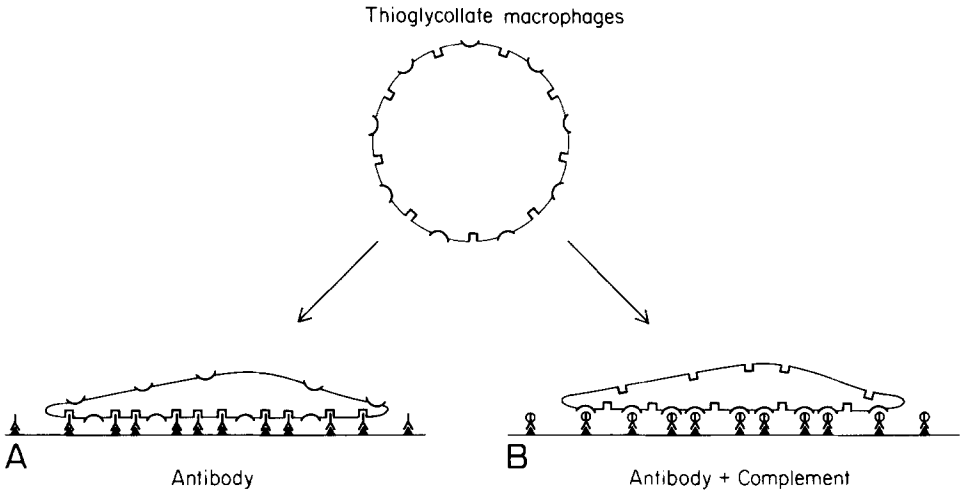

FIG. 4. Model for receptor modulation in thioglycollate-elicited macrophages: thioglycollate-elicited macrophages seeded onto immobilized antibody-antigen complexes ( ) modulate their Fc receptors ( ) while their complement receptors ( ) remain unperturbed, (A) of the drawing. Experimentally, this is indicated by a loss of attachment and ingestion of E(IgG) on the nonadherent membrane surface, while the macrophages continue to bind and phagocytize E(IgM)C. (B), the binding of complement ( ) onto the immobilized immune complexes results in the modulation of the macrophages' complement (C3b) receptors from the cells' nonadherent plasma membrane. Complement masks the Fc domain of IgG ( ) so that it cannot be recognized by the cells Fc receptors leaving them available throughout the plasma membrane. This is shown experimentally by the ability of thioglycollate-elicited macrophages to bind and ingest E(IgG) upon plating onto antibody-antigen-and-complement-coated coverslips.

Scharfstein et al., (43) have shown that when antibody-antigen complexes are coated with complement they no longer bind staphylococcal protein A. The amount of inhibition of staphylococcal protein A binding was proportional to the amount of complement fixed by the complexes. Because staphylococcal protein A binds to the Fc portion of IgG (48) their results provide direct evidence that complement components mask the Fc segment.

These findings raise two issues relevant to immunopathology: first, they suggest that in vivo, where complement is present in abundance, the Fc segments of IgG in immune complexes will be unavailable for binding to the Fc receptors of lymphocytes, macrophages, and polymorphonuclear leukocytes. Under these circumstances many of these cells' effector functions may be mediated principally by their complement receptors, and not by their Fc receptors. Second, they indicate a possible function for subclasses of IgG that do not fix complement. Particle- or antigen-bound noncomplement fixing antibodies should retain the capacity to interact with leukocyte Fc receptors in the presence of complement, and thereby to mediate a specific subset of immune effector functions.

Summary

We have examined the Fc- and complement-receptor function of resident and thioglycollate-elicited mouse peritoneal macrophages plated on surfaces coated with rabbit antibody-antigen complexes and with complement. We derive four major conclusions from these studies. (*a*) The trypsin-resistant Fc receptors of resident and

thioglycollate-elicited macrophages are completely modulated when these cells are plated on rabbit antibody-antigen complexes. Residual Fc receptor activity is a result of the incomplete modulation of trypsin-sensitive IgG2a receptors. (*b*) The complement receptors of thioglycollate-elicited macrophages, but not of resident macrophages, are modulated when these cells are plated on complement-coated surfaces. The capacity of the two cell types to modulate their complement receptors is correlated with their ability to ingest complement-coated erythrocytes. (*c*) The complement and Fc receptors of both types of macrophages move independently of one another. (*d*) Complement masks the Fc segments of IgG in immune complexes thereby rendering them ineffective as ligands for macrophage Fc receptors.

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