AUGMENTATION OF MACROPHAGE COMPLEMENT RECEPTOR FUNCTION IN VITRO

III. C3b Receptors That Promote Phagocytosis Migrate within the Plane of the Macrophage Plasma Membrane*

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The process of immunologically mediated phagocytosis can be divided into three steps: (*a*) attachment of a ligand-coated particle to specific receptors on the phagocytic cell's plasma membrane; (*b*) generation and transmission of a phagocytic signal by receptor-ligand engagement; and (*c*) the ingestion process per se (1). Depending upon the receptor engaged and the physiologic state of the phagocytic cell, the process may or may not proceed to completion. For example, engagement of macrophage Fc receptors by particle-bound IgG virtually always leads to particle ingestion (2–5). In contrast, engagement of macrophage complement receptors by particle-bound C3b, which always mediates efficient particle binding (2–8), promotes particle ingestion only by macrophages that have been physiologically altered (5–8).

Recent studies by Michl et al. (9, 10) suggest that for a receptor to promote particle ingestion, it must be able to move within the plane of the cell's plasma membrane. The Fc receptors of both resident and thioglycollate-elicited mouse peritoneal macrophages mediate both attachment and ingestion of IgG-coated particles (2, 3, 6). The C3b receptor of resident macrophages mediates only attachment of C3b-coated particles (2, 3), whereas the C3b receptor of thioglycollate-elicited macrophages promotes ingestion as well (6). Michl et al. (9) found that when macrophages were plated on coverslips coated with antigen-antibody complexes, the Fc receptor activity of both resident and thioglycollate-elicited macrophages disappeared from the nonadherent surface of the cells. When macrophages were plated on coverslips coated with antigen-antibody-complement complexes, the C3b receptor activity of thioglycollate-elicited macrophages also disappeared, but that of resident macrophages did not. Subsequent studies (10) provided indirect evidence that the disappearance of receptor activity from the nonadherent macrophage surface was the result of receptor migration within the plane of the macrophage plasma membrane to the immobilized ligand. The correlation of the ability of these receptors to redistribute on the macrophage surface with their ability to promote phagocytosis suggested that receptor mobility may be a prerequisite for particle ingestion.

We have previously reported that treatment with a unique lymphokine enables resident mouse peritoneal macrophages to phagocytize via their complement receptors

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(7, 8) and have presented evidence that the lymphokine acts by enabling complement receptor engagement by C3b ligands to generate a phagocytic signal, thereby linking the cell surface binding event with the intracellular phagocytic machinery (8). In the present experiments, we used techniques similar to those of Michl et al. (9, 10) to study the topography of C3b receptors of resident mouse peritoneal macrophages treated with the lymphokine. Our results indicate that lymphokine treatment enables the macrophages' C3b receptors to migrate within the plane of the cells' plasma membrane and that manipulations of macrophages that abrogate one response to the lymphokine, complement receptor mobility, also abrogate the other response, complement receptor-mediated phagocytosis. These findings strongly suggest that lateral mobility of a ligand-bound receptor within the macrophage plasma membrane is an essential component of the phagocytic signal. Moreover, our results indicate that the difference in complement receptor function among various populations of macrophages is not due to the expression of different types of complement receptors by the different macrophage populations but rather to a difference in the relationship of the C3b receptor with other plasma membrane or intracellular components.

Materials and Methods

Reagents and Media. Glycine, bovine serum albumin (BSA),[1] colchicine, vinblastine, and cyclohexamide were obtained from Sigma Chemical Co., St. Louis, Mo.; chromerge, glutaraldehyde, 50% aqueous solution, and concentrated sulfuric acid were purchased from Fisher Scientific Co., Atlanta, Ga.; medium 199 was purchased from Microbiological Associates, Walkersville, Md.; fetal bovine serum (FBS) and penicillin-streptomycin were obtained from Grand Island Biological Company, Grand Island, N. Y.; poly-L-lysine (PLL) was purchased from Miles Laboratories, Elkhart, Ind.; and Brewer thioglycollate medium was obtained from Difco Laboratories, Detroit, Mich. FBS was heat inactivated (56°C for 30 min) before use.

Lyophilized rabbit anti-BSA IgG was obtained from N. L. Cappel Laboratories, Inc., Cochranville, Pa. It was reconstituted at 6 mg/ml of antibody protein according to the manufacturer's directions in phosphate-buffered saline (PBS), pH 7.4, and was further diluted in medium for use in experiments as indicated. Sheep erythrocytes (E) in Alsever's solution, obtained from Scott Laboratories, Fiskeville, R. I., were washed three times in PBS without Mg^{++} and Ca^{++} (PD) and resuspended in medium. A 4.05% aqueous solution of Brewer thioglycollate medium was prepared according to the manufacturer's directions. Lumicolchicine was prepared in PBS as previously described (11). Taxol was a generous gift from Dr. J. Smith, University of Alabama in Birmingham.

Immunologically Coated Sheep E. Rabbit anti-sheep E IgM and rabbit anti-sheep E IgG were obtained from Cordis Laboratories, Miami, Fla. Sheep E coated with anti-E IgG [E(IgG)] or with anti-E IgM and the first four complement components [E(IgM)C] were prepared as previously described (3).

Animals. Peritoneal macrophages and mouse serum used to prepare immobilized immune complexes were obtained from 20–30 g female, CD-1 Swiss mice (Charles River Breeding Laboratories, Inc., Wilmington, Mass.). C5-deficient mouse serum was obtained from female, C5-deficient AKR mice (The Jackson Laboratory, Bar Harbor, Maine). Both AKR and CD-1 mouse serum were adsorbed twice at 4°C for 15 min with 20% sheep E before being frozen in small aliquots at −70°C. Serum was thawed on ice just before use as a complement source.

Macrophages. Resident mouse peritoneal macrophages were obtained by peritoneal lavage and cultured (2×10^5/well) in medium in 24-well tissue culture clusters (Costar Bellco Glass, Inc., Vineland, N. J.). Macrophages elicited with thioglycollate medium were harvested 4 d

[1] *Abbreviations used in this paper:* BSA, bovine serum albumin; BSA-anti-BSA-C, complement-containing BSA-anti-BSA complexes; C3b INA, C3b inactivator; E, erythrocytes; E(IgG), E coated with anti-E IgG; E(IgM)C, E coated with anti-E IgM and the first four complement components; FBS, fetal bovine serum; PBS, phosphate-buffered saline; PD, PBS without Ca^{++} or Mg^{++} ions; PLL, poly-L-lysine.

after intraperitoneal injection of 1 ml/mouse of thioglycollate medium and cultured (1.25×10^5/well) in the same manner as resident peritoneal macrophages.

Preparation of Coverslips. Immobilized immune complexes were fixed onto 13-mm glass coverslips as previously described (9). Coverslips were cleaned overnight at room temperature with 1% chromerge in concentrated sulfuric acid, washed for 2–4 h in running water, rinsed with distilled water, dried, and stored at room temperature. They were then incubated in PD containing 0.1 mg/ml of PLL for 30 min at room temperature, washed in PD, incubated for 15 min at room temperature in 2.5% glutaraldehyde in PD, washed with distilled water and then with PD, and reacted for 30 min at room temperature with 3 mg/ml of BSA in PD. The reaction with BSA was stopped by overnight incubation at room temperature in 0.2 M glycine in PD, after which coverslips were either used immediately or stored at 4°C. Before further treatment, coverslips were washed twice with PD and placed into 24-well cluster plates.

To prepare immobilized immune complexes, 100 μg of anti-BSA IgG in 0.5 ml of medium was added to each well, coverslips were incubated for 30 min at room temperature, washed with PD, and used the same day. To prepare complement-containing complexes (BSA-anti-BSA-C), neat fresh mouse serum was added directly to the wells (75 μl/well) containing coverslips coated with BSA-anti-BSA complexes. Coverslips were incubated for 10 min at 37°C, washed with PD, and used the same day.

Preparation of Lymphokine. Culture supernates containing the lymphokine that enables resident mouse peritoneal macrophages to ingest C3b-coated particles were prepared and used as previously described (7, 8).

Inhibition of Macrophage Protein Synthesis. Macrophage protein synthesis was blocked by incubating the cells with 5 μg/ml of cyclohexamide for 1 h at 37°C, treatment that we have previously shown inhibits protein synthesis by >85% (8).

Assessment of Macrophage Fc and C3b Receptor Function. After a 1-h incubation at 37°C to permit spreading of macrophages on coverslips, monolayers were washed twice with PBS and overlaid with 1 ml of either medium or lymphokine. After a 15-min incubation at 37°C, some cultures were washed and covered with 1 ml of fresh medium; others were not washed. 0.1 ml of 0.5% E(IgG) or E(IgM)C was added to the wells and cultures were incubated for 1 h at 37°C. The cultures were then washed twice with PBS and fixed with 2.5% glutaraldehyde in 0.1 M cacodylate buffer, and E attachment and ingestion were scored by phase-contrast microscopy.

Miscellaneous. Viability of macrophages plated on coverslips coated with immune complexes was assessed by their ability to exclude 0.4% trypan blue dye after 10 min at room temperature. Protein concentrations were determined by the method of Lowry et al. (12), using BSA as a standard.

Presentation of Results. Each result reported is the average of at least three separate determinations, each performed in duplicate. The attachment index and the phagocytic index are the number of E attached or ingested by 100 macrophages and were obtained by multiplying the percent of macrophages that had attached or ingested any E by the average number of E attached or ingested per macrophage. Each result is presented as mean ± SEM.

Results

Effects of Immobilized Immune Complexes on the Topography of Macrophage C3b Receptors. Resident and thioglycollate-elicited macrophages were plated on coverslips, some of which were coated with immune complexes. Some resident macrophages were treated with lymphokine, and each population was incubated with E(IgM)C (Table I). All macrophages plated on coverslips that had received no treatment avidly bound E(IgM)C; thioglycollate-elicited and lymphokine-treated macrophages also ingested E(IgM)C, whereas resident macrophages did not (Table I, line 1). The abilities of macrophages to bind and to ingest E coated with C3b were not affected by plating the cells on coverslips coated with either PLL and BSA or PLL and BSA-anti-BSA (Table I, lines 2 and 3). The complement receptor activity of resident macrophages plated on BSA-anti-BSA-C was not reduced (Table I, line 4, column 2).

TABLE I

Interaction of Mouse Peritoneal Macrophages with E(IgM)C

Coverslip treatment	Macrophages*					
	Resident		Thioglycollate-elicited		Lymphokine-treated	
	PI	AI	PI	AI	PI	AI
None	0	538 ± 18	331 ± 85	667 ± 21	113 ± 13	581 ± 14
PLL-BSA	0	603 ± 54	398 ± 134	779 ± 96	122 ± 6	562 ± 39
PLL-BSA-anti-BSA	0	590 ± 37	337 ± 97	680 ± 89	114 ± 10	596 ± 7
PLL-BSA-anti-BSA-C	0	638 ± 32	2 ± 1	13 ± 2	0	21 ± 4

* PI, phagocytic index; AI, attachment index.

However, the abilities of both thioglycollate-elicited and lymphokine-treated macrophages to bind and to ingest E(IgM)C was abolished by plating them on complement-containing complexes (Table I, line 4, columns 3–6). In parallel experiments, we confirmed that resident, thioglycollate-elicited, and lymphokine-treated macrophages plated on coverslips coated with BSA-anti-BSA complexes lost the ability to bind and ingest E(IgG) (data not shown).

These results indicate that C3b receptors of macrophages that are capable of phagocytizing complement-coated erythrocytes disappear from the nonadherent surface of macrophages plated on complement-coated immune complexes, whereas C3b receptors of macrophages that are incapable of phagocytizing complement-coated E cannot be modulated by complement-containing complexes. It has been suggested that receptor disappearance is a consequence of receptor sequestration on immobilized ligands (9, 10). If that is the mechanism here, then these findings suggest that the lymphokine acts by freeing anchored macrophage C3b receptors so that they can move randomly within the plane of the macrophage plasma membrane and become trapped on immobilized C3b ligands.

Effect of Lymphokine Preincubation on the Modulation of Macrophage C3b Receptors. In the above experiments, the complement receptor function of lymphokine-treated macrophages was assayed in the presence of lymphokine. We reasoned that if lymphokine treatment freed previously anchored C3b receptors so that they could move within the plasma membrane and become sequestered on complement-containing, immobilized immune complexes, then the continued presence of lymphokine was probably unnecessary, for once receptors were bound to complexes, they probably could not migrate back to the nonadherent macrophage surface. The following experiments were designed to examine the necessity for continuous lymphokine treatment. At the same time, we determined the rate at which complement receptor activity was modulated by the lymphokine.

When macrophages were incubated with lymphokine and E(IgM)C at 37°C in the preceding experiments, four separate events were occurring simultaneously. The lymphokine was exerting its effect on macrophage complement receptors, complement receptors may have been moving within the plane of the macrophage plasma membrane, E(IgM)C were settling onto macrophage monolayers, and macrophage complement receptors were interacting with E(IgM)C. To determine the rate of complement receptor modulation independently of E(IgM)C settling and binding, we used the following protocol. Macrophages plated on BSA-anti-BSA-C complexes

were incubated with lymphokine at 37°C for various periods of time. Cultures were washed twice with ice-cold medium and E(IgM)C in ice-cold medium were added. Cultures were incubated either at 4°C for 1 h or at 4°C for the first 15 min and then at 37°C for the final 45 min. Because the lymphokine cannot act (8) and complement receptor activity cannot be modulated at 4°C (data not shown), both the time during which the lymphokine could act and the time during which complement receptor modulation could occur were limited to the initial 37°C incubation. E(IgM)C binding could then be assessed at 4°C, independently of these events. The subsequent 45-min, 37°C incubation was intended to allow time for recovery of macrophage complement receptors from the effects of lymphokine treatment.

The results presented in Fig. 1 were obtained by incubating macrophages with E(IgM)C first at 4°C for 15 min and then at 37°C for 45 min; identical kinetics were obtained when macrophages were incubated with E(IgM)C at 4°C for 1 h. Loss of complement receptor activity from the nonadherent macrophage surface was apparent after <2 min of lymphokine treatment, and the maximal lymphokine effect was achieved by 5 min. Thus, the lymphokine modulates macrophage complement receptor function very rapidly. Moreover, because complement receptor activity did not return to the nonadherent macrophage surface during the 45-min, 37°C incubation after lymphokine removal, the continued presence of the lymphokine is not necessary to maintain complement receptor modulation. These findings are consistent with the receptors' being sequestered on immobilized immune complexes.

Recovery of C3b Receptor Activity after Modulation by the Lymphokine. In the following experiments, we explored further the recovery of macrophage complement receptors from the effects of lymphokine treatment. Macrophages plated on BSA-anti-BSA-C complexes were treated with lymphokine for 15 min at 37°C, washed, covered with either medium or cyclohexamide, and replaced at 37°C. At various times thereafter, macrophage complement receptor function was assessed (Fig. 2). Lymphokine-treated macrophages recovered complement receptor binding activity completely after a 4-h incubation in medium. Recovery of receptor activity required macrophage protein synthesis, for it did not occur in the presence of cyclohexamide. When cyclohexamide was removed, macrophages regained receptor activity at the same rate as those not

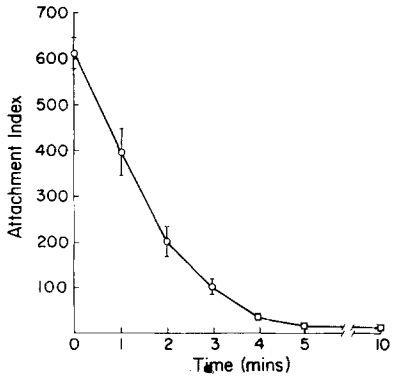

FIG. 1. Effect of lymphokine preincubation on the modulation of macrophage C3b receptors. Macrophages plated on BSA-anti-BSA-C complexes were treated with lymphokine for the periods of time indicated on the abscissa, after which they were incubated with E(IgM)C at 4°C for 15 min and then at 37°C for 45 min.

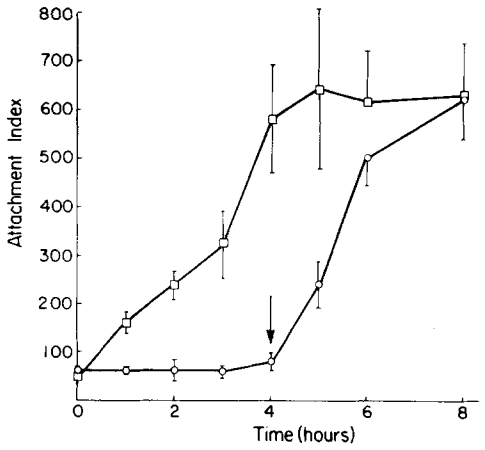

FIG. 2. Recovery of C3b receptor activity after modulation by the lymphokine. Macrophages plated on BSA-anti-BSA-C complexes were treated with lymphokine for 15 min at 37°C, then incubated in either medium alone (□) or in medium containing 5 μg/ml of cyclohexamide (○). After a 4-h incubation with the drug, cyclohexamide-treated macrophages were washed and covered with medium (arrow). At the times indicated on the abscissa, the cells' ability to bind E(IgM)C was determined.

treated with the drug, indicating that cyclohexamide treatment was not cytotoxic. These results suggest that the return of complement receptor activity to the nonadherent surface of lymphokine-treated macrophages was dependent upon the synthesis of new complement receptors and their insertion into the macrophage plasma membrane.

Effect of Continuous Lymphokine Treatment on the Recovery of Macrophage C3b Receptor Activity. Macrophages plated on BSA-anti-BSA-C complexes were treated with lymphokine. Some cultures were washed after 15 min and covered with fresh medium; others were left in lymphokine-containing medium for up to 24 h. As shown in Fig. 3, all lymphokine-treated macrophages lost complement receptor activity. Those treated for only 15 min recovered activity within 4 h of removal of the lymphokine. However, the continuous presence of lymphokine completely inhibited the reappearance of complement receptor activity on the nonadherent macrophage surface. Also shown in Fig. 3, thioglycollate-elicited macrophages plated on BSA-anti-BSA-C-coated coverslips and incubated in medium similarly failed to recover complement receptor function. These results suggest that in macrophages whose complement receptors are unable to migrate within the plasma membrane, newly synthesized receptors inserted into the nonadherent macrophage surface remain there and receptor activity is recovered within a few hours. In contrast, in macrophages whose complement receptors are able to move within the plasma membrane, newly synthesized receptors inserted into the nonadherent macrophage surface quickly migrate to the adherent cell surface and are trapped there, preventing recovery of receptor activity on the nonadherent macrophage surface.

Release of Bound Complement Receptors from Immobilized Immune Complexes. To prove that migration to and sequestration on immobilized ligands was the mechanism by which receptors disappeared from the nonadherent surface of macrophages plated on immune complexes, we needed to demonstrate that complement receptors could be

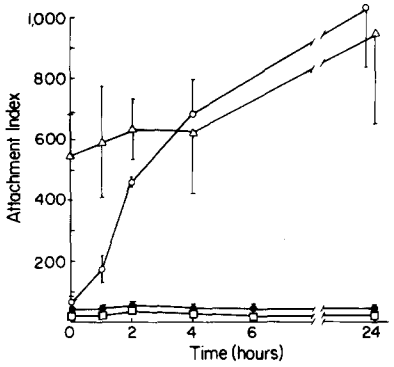

FIG. 3. Effect of continuous lymphokine treatment on the recovery of macrophage C3b receptor activity. Macrophages were plated on BSA-anti-BSA-C complexes. Some macrophages (○ and ●) were treated with lymphokine; others (△) were not. Some macrophages (●) were incubated with lymphokine for the periods of time indicated on the abscissa. Others (○) remained in lymphokine for only 15 min, after which they were incubated in medium alone for the periods of time indicated on the abscissa. Thioglycollate-elicited macrophages (□) were plated on BSA-anti-BSA-C complexes and incubated in medium alone for the periods of time indicated on the abscissa. At the conclusion of the incubations, the ability of macrophages to bind E(IgM)C was determined.

released from the immobilized ligands and could redistribute over the macrophage surface. Preliminary experiments indicated that pretreatment of BSA-anti-BSA-C-coated coverslips with heat-inactivated (56°C for 30 min) mouse serum, but not with fresh mouse serum, markedly altered the complexes. Macrophages plated on these coverslips and treated with lymphokine retained their ability to bind E(IgM)C (data not shown), suggesting that C3b inactivator (C3b INA) in the serum cleaved C3b ligands from immobilized complexes. We reasoned that C3b INA in heat-inactivated serum might also cleave complement receptor-bound C3b from immobilized immune complexes, thereby releasing the bound complement receptors.

Macrophages plated on BSA-anti-BSA-C complexes were treated with lymphokine for 15 min, lymphokine was removed, and cultures were incubated for 45 min at 37°C with either fresh medium alone or with medium containing 30% heat-inactivated mouse serum. Lymphokine-treated macrophages incubated in medium alone were unable to bind E(IgM)C (Fig. 4, bar B), whereas those in cultures subjected to treatment with heat-inactivated mouse serum (Fig. 4, bar C) were as capable of binding E(IgM)C as were macrophages that had been plated on BSA-anti-BSA-C-coated coverslips and not subjected to lymphokine treatment (Fig. 4, bar A). Similarly, thioglycollate-elicited macrophages, which lost virtually all complement receptor activity when plated on BSA-anti-BSA-C-coated coverslips (Table I), regained receptor activity when treated with heat-inactivated mouse serum (data not shown).

It was possible that the rapid recovery of complement receptor activity by macrophages treated with heat-inactivated mouse serum was a result of the serum's enhancing the cells' ability either to synthesize new complement receptors or to insert complement receptors into their plasma membranes. To test these possibilities, we performed experiments similar to those above, but using either fresh mouse serum, which contains both C3b INA and C3b-generating capability and which therefore would generate and deposit new C3b molecules on BSA-anti-BSA complexes at the same time that it destroyed the previously bound C3b molecules, or heat-inactivated

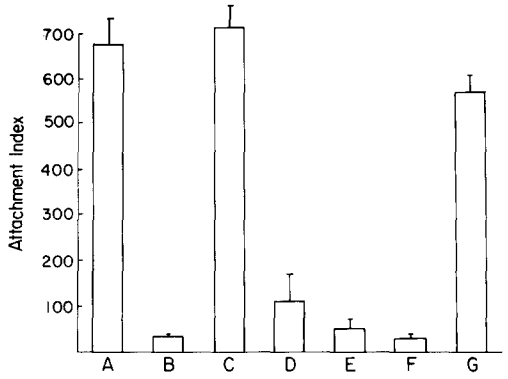

FIG. 4. Release of bound complement receptors from immobilized immune complexes. Macrophages plated on BSA-anti-BSA-C complexes were either incubated in medium alone (bar A) or treated with lymphokine for 15 min at 37°C (bars B–E). They were then washed and treated with either heat-inactivated mouse serum (bar C), fresh mouse serum (bar D), or heat-inactivated FBS (bar E) for 45 min at 37°C. Some macrophages were incubated at 37°C sequentially with 5 μg/ml of cyclohexamide for 1 h, with lymphokine for 15 min, and with either medium alone (bar F) or heat-inactivated mouse serum (bar G) for 45 min, cyclohexamide being included in the latter two incubations as well. At the conclusion of the incubations, macrophages were washed and covered with fresh medium and their ability to bind E(IgM)C was determined.

FBS, serum that contains "nutritional factors" similar to those of mouse serum but contains C3b INA that we speculated might be incapable of recognizing mouse C3b.[2] Both of these sera should provide components and activities similar to those of heat-inactivated mouse serum, but neither should cause a net loss of C3b ligands from immobilized immune complexes. Treatment with these sera did not restore complement receptor activity to the nonadherent macrophage surface (Fig. 4, bars D and E).

In other experiments, macrophages plated on BSA-anti-BSA-C complexes were treated at 37°C sequentially with cyclohexamide for 1 h, with lymphokine for 15 min, and with either medium alone or 30% heat-inactivated mouse serum for 45 min, cyclohexamide being included in the latter two incubations as well. Macrophages treated with cyclohexamide and then with lymphokine did not bind E(IgM)C (Fig. 4, bar F); in contrast, macrophages treated with cyclohexamide and lymphokine and then with heat-inactivated mouse serum were able to bind E(IgM)C normally (Fig. 4, bar G). Thus, the lymphokine modulates, and heat-inactivated mouse serum restores, complement receptor activity by mechanisms that are independent of macrophage protein synthesis.

These results exclude the possibility that the rapid recovery of complement receptor function by macrophages treated with heat-inactivated mouse serum was the result of either enhanced synthesis of complement receptors or of an enhanced rate of insertion of complement receptors into the macrophage plasma membrane. They strongly suggest that C3b INA and proteases in heat-inactivated mouse serum cleaved immune complex-bound C3b, thereby releasing C3b receptors from the immobilized ligands and permitting them to return to their former positions over the entire macrophage surface. Thus, the results provide strong support for the hypothesis that the mechanism by which complement receptors disappear from the nonadherent surface of macro-

[2] We are unaware of any information regarding the species specificity of bovine C3b inactivator.

phages plated on immune complexes and treated with lymphokine is by migrating to and becoming sequestered on immobilized C3b ligands.

Further Definition of the Mechanism of Action of the Lymphokine. Previous work from this laboratory (13) has shown that treatment of macrophages with pharmacologically achievable concentrations of hydrocortisone completely abolishes the cells' ability to respond to the lymphokine and phagocytize via their complement receptors. To determine whether hydrocortisone treatment also inhibits the lymphokine's effect on complement receptor mobility, we treated macrophages plated on BSA-anti-BSA-C complexes with 10^{-8} M hydrocortisone for 1 h at 37°C, washed the cells with medium, treated them with lymphokine for 15 min at 37°C, and determined their interaction with E(IgM)C. These macrophages bound E(IgM)C normally (Fig. 5, bar C), indicating that hydrocortisone abrogates the effect of the lymphokine on macrophage complement receptor mobility and suggesting that the lymphokine enables complement receptors to move within the macrophage plasma membrane by the same mechanism as it enables them to promote phagocytosis.

To explore further the mechanism by which the lymphokine acts on macrophage complement receptors, we treated macrophages plated on BSA-anti-BSA-C complexes with either 10^{-6} M colchicine or 10^{-6} M vinblastine for 1 h at 37°C, then treated them with lymphokine for 15 min at 37°C, maintaining the drug in this incubation mixture as well. Macrophages were washed and their interaction with E(IgM)C was assessed. These macrophages avidly bound E(IgM)C (Fig. 5, bars D and E). That the drugs achieved their effect by depolymerizing the macrophages' microtubules was indicated by the ineffectiveness of lumicolchicine (Fig. 5, bar F), a photoisomer that retains many of the effects of colchicine but does not bind tubulin or depolymerize microtubules (14), and by the ability of taxol, a microtubule-stabilizing agent (15–17), to prevent the effect of colchicine (Fig. 5, bar G). Colchicine pretreatment did not prevent the disappearance of complement receptor activity from the nonadherent surface of thioglycollate-elicited macrophages when they were plated on BSA-anti-

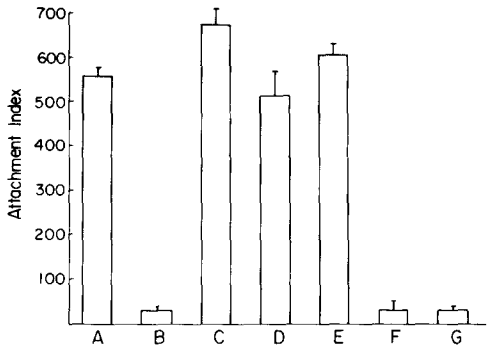

FIG. 5. Effects of hydrocortisone and of microtubule depolymerization on the effect of the lymphokine on macrophage complement receptor mobility. Macrophages plated on BSA-anti-BSA-C complexes were either incubated in medium alone (bar A) or treated with lymphokine for 15 min at 37°C (bars B–G). Some macrophages were pretreated either with 10^{-8} M hydrocortisone for 1 h at 37°C (bar C), or with 10^{-6} M colchicine (bar D), 10^{-6} M vinblastine (bar E), or 10^{-6} M lumicolchicine (bar F) for 2 h at 37°C before lymphokine treatment. Some macrophages were pretreated with 5×10^{-6} M taxol for 1 h at 37°C and then with 10^{-6} M colchicine for 2 h at 37°C before lymphokine treatment (bar G). At the conclusion of the incubations, macrophages were washed and covered with fresh medium and their ability to bind E(IgM)C was determined.

BSA-C complexes (data not shown), indicating that intact microtubule function is not required for complement receptor mobility per se. The inhibition of lymphokine-induced complement receptor modulation by colchicine and vinblastine indicates, therefore, that intact microtubule function is necessary for macrophages to respond to the lymphokine.

Discussion

We considered four mechanisms by which plating macrophages on immobilized immune complexes might cause the disappearance of receptor activity from the nonadherent macrophage surface:

(*a*) Immune complexes might detach from the glass surface and bind to receptors over the entire macrophage surface. This seems unlikely to account for our findings, however, because soluble immune complexes, which can very efficiently block macrophage Fc receptors, appear to be unable to block macrophage C3b receptors, even when they contain complement (13, 18). In addition, Rabinovitch et al. (19) and Michl et al. (9) have demonstrated that during several hours of incubation at 37°C virtually no immune complexes detach from the coverslips and bind to the nonadherent macrophage surface.

(*b*) Engagement by immobilized ligands of receptors on the adherent surface of the mononuclear phagocyte triggers a number of metabolic events that result in release into the culture medium of neutral proteases (20), lysosomal enzymes (20), toxic products of oxygen metabolism (21), and probably prostaglandins (22), any of which might damage receptors on the nonadherent macrophage surface. Such a mechanism would not explain the selectivity of receptor inactivation seen, for example, in macrophages plated on antigen-antibody complexes; whereas their Fc receptor activity was virtually nil, their complement receptor activity was normal (9). Moreover, Ragsdale and Arend (23) have presented strong evidence against the participation of either neutral proteases, lysosomal hydrolases, H_2O_2, superoxide anion, or prostaglandins in receptor inactivation.

(*c*) Engagement of immobilized ligands by receptors on the adherent macrophage surface might initiate intracellular metabolic events that cause changes in either the number or the function of identical receptors on the nonadherent macrophage surface. Ragsdale and Arend (23) have, in fact, presented evidence that supports a cAMP-mediated reaction leading to the abolition of Fc receptor activity from the nonadherent surface of human monocytes plated on immune complexes. These investigators proposed that engagement of Fc receptors on the adherent monocyte surface led either to phosphorylation of the remaining Fc receptors or to activation of phospholipases that inactivated Fc receptors located on the nonadherent surface of the cells. This formulation does not readily explain the selectivity of receptor inactivation. Moreover, Michl et al. (10) have found that mouse peritoneal macrophages plated on antigen-antibody complexes bind <25% as much monoclonal anti-Fc receptor antibody as their counterparts plated on coverslips without complexes. Thus, Fc receptors on the nonadherent macrophage surface are more than functionally altered; they are either severely modified antigenically or absent from that macrophage surface.

(*d*) All, or nearly all, receptors might migrate to the adherent surface of macrophages plated on immune complexes, leaving the nonadherent surface both functionally and physically devoid of receptors. It has been argued that receptor mobility

within the macrophage plasma membrane is an unlikely explanation for these findings because pharmacologic agents that inhibit capping of certain surface antigens by some cells do not prevent the disappearance of receptor activity from the macrophage surface (23). Capping is the surface aggregation of plasma membrane molecules that is induced by the binding of a multivalent ligand and that is under the control of certain intracellular structures, e.g., actin filaments (24–31). Our finding that complement receptor activity reappeared on the nonadherent macrophage surface promptly after treatment of immune complex-bound, lymphokine-treated macrophages with C3b INA (Fig. 4) strongly suggests that all C3b receptors had been sequestered on immobilized ligands and that they were freed to redistribute over the macrophage surface once the C3b ligand was destroyed. We therefore believe that receptor movement within the macrophage plasma membrane does account for the experimental findings, but we think that the movement involved is not capping but rather the random mobility of receptor molecules in the plasma membrane's fluid bilayer (25, 32, 33). This mechanism most reasonably deals with all experimental findings. It explains the selectivity of receptor inactivation. It also explains how Fc receptor activity of all mononuclear phagocytes (23; and S. C. Silverstein, personal communication) and complement receptor activity of thioglycollate-elicited macrophages (Fig. 3), once modulated, fail to reappear on the nonadherent cell surface, whereas complement receptor activity returns to the nonadherent surface of lymphokine-treated macrophages within 4 h of lymphokine removal. Fc receptors are intrinsically able to move within the plane of the macrophage plasma membrane (10). Thus, in macrophages plated on immune complexes, whenever a newly synthesized Fc receptor is inserted into the plasma membrane, it wanders randomly within the membrane, finds its way to the adherent macrophage surface, and is bound there. Fc receptor activity cannot return to the nonadherent macrophage surface until immobilized IgG ligands have been saturated with Fc receptors. Similar arguments probably explain the failure of complement receptor activity to reappear on the nonadherent surface of thioglycollate-elicited macrophages plated on complement-containing, immobilized immune complexes. Complement receptor activity does not disappear from the nonadherent surface of resident peritoneal macrophages plated on antigen-antibody-complement complexes, presumably because, like the C3b receptors of human neutrophils (34), these receptors are normally fixed within the macrophage plasma membrane. Treatment of resident macrophages with the lymphokine that augments their complement receptor function releases complement receptors from their plasma membrane anchors and permits them to move randomly within the membrane. As they encounter immobilized C3b ligands on the immune complex substrate, they are trapped on the adherent macrophage surface. As newly synthesized complement receptors are inserted into the macrophage plasma membrane, they similarly wander to the adherent cell surface and become trapped on the immobilized complexes. If this explanation is correct, complement receptor activity should not reappear on the nonadherent surface of immune complex-bound macrophages so long as they are in the presence of lymphokine; however, receptor activity should progressively reappear after lymphokine is removed. As shown in Fig. 3, this is precisely the case.

Previous studies have indicated that whereas macrophages were able to phagocytize E(IgM)C in the presence of the lymphokine (8), macrophages preincubated with the

lymphokine for up to several hours were unable to ingest E(IgM)C after lymphokine removal (7). In light of the results obtained here with regard to modulation of complement receptor topography by the lymphokine, these previous results suggest that the normally anchored C3b receptors are freed by the lymphokine to move randomly in the plane of the plasma membrane. If engaged by C3b ligands while in the presence of the lymphokine, the receptors become functionally linked to the cell's phagocytic machinery, leading to phagocytosis of particles coated with C3b. If not engaged by C3b ligands while the lymphokine is present, they return promptly to their anchored state when the lymphokine is removed.

Many plasma membrane receptors are mobile in either their free or their ligand-bound states (35–40); and certain receptors, when engaged by their ligands, must move laterally within the plasma membrane for ligand-receptor binding to be translated into altered cell behavior or metabolism (35, 36, 38). In some cases, lateral mobility of receptors serves to link them with intramembrane second messenger molecules (35–38). In others, aggregation of ligand-bound receptors with each other is required to generate an intracellular response (26, 28–30, 37), and receptor aggregation may result in the physical linking of receptors with intracellular structures. For example, β_2-microglobulin, aminopeptidase, Na^+,K^+-ATPase, B lymphocyte surface immunoglobulin, and concanavalin A receptors are normally freely mobile within the plasma membrane but become fixed to actomyosin when bound by their specific antibodies or lectin (25, 26, 28–30).

Results of several studies (9, 13, 19, 23, 40, 41, and this paper) demonstrate a precise correlation between the ability of receptors to redistribute on the macrophage surface and their ability to promote phagocytosis (Table II), suggesting that receptor mobility may be an essential component of the phagocytic signal. We propose the following model for the initiation and transmission of that signal. Receptors that are wandering

TABLE II

Correlation between the Mobility of Fc and C3b Receptors on the Mononuclear Phagocyte's Surface and the Receptors' Ability to Promote Phagocytosis

Mononuclear phagocyte	Fc Receptor		C3b Receptor		References
	Mobility	Phagocytosis	Mobility	Phagocytosis	
Human monocyte	+	+	−	−	23, 40
Resident mouse peritoneal macrophage	+	+	−	−	9, 19, 41, this paper
Thioglycollate-elicited mouse peritoneal macrophage	+	+	+	+	9, this paper
Lymphokine-treated mouse peritoneal macrophage	+	+	+	+	This paper
Hydrocortisone-pretreated, lymphokine-treated mouse peritoneal macrophage	+	+	−	−	13, this paper
Colchicine-pretreated, lymphokine-treated mouse peritoneal macrophage*	+	+	−	−	This paper

* Colchicine's inhibition of the lymphokine's effect on macrophage complement receptor mobility is shown in Fig. 5. Data for the effects of colchicine on Fc receptor mobility and on Fc receptor- and C3b receptor-mediated phagocytosis are not shown.

randomly within the macrophage plasma membrane become engaged by appropriate particle-bound ligands. It may be that ligand engagement of the mobile receptors initiates the phagocytic signal by directing the individual receptors to an intramembrane second messenger molecule. Alternatively, the ligand-bound receptors may first aggregate on the cell surface and then become functionally and perhaps physically linked with the intracellular phagocytic machinery, and actin and its regulatory proteins (42, 43). Changes in the physical state of actin then provide the motive force for ingestion of the ligand-coated particle.

Summary

We have previously reported that treatment with a unique lymphokine enables resident mouse peritoneal macrophages to phagocytize via their complement receptors and we have presented evidence that the lymphokine acts by enabling complement receptor engagement by C3b ligands to generate a phagocytic signal, thereby linking the cell surface binding event with the intracellular phagocytic machinery. In the present experiments, we used immobilized immune complexes to study the topography of C3b receptors of resident mouse peritoneal macrophages treated with the lymphokine. Our results indicate that lymphokine treatment enables the macrophages' C3b receptors to migrate within the plane of the cells' plasma membrane and that manipulations of macrophages that abrogate one response to the lymphokine, complement receptor mobility, also abrogate the other response, complement receptor-mediated phagocytosis. These findings strongly suggest that lateral mobility of a ligand-bound receptor within the macrophage plasma membrane is an essential component of the phagocytic signal. Moreover, our results indicate that the difference in complement receptor function among various populations of macrophages is not due to the expression of different types of complement receptors by the different macrophage populations but rather to a difference in the relationship of the C3b receptor with other plasma membrane or intracellular components.

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