AUGMENTATION OF MACROPHAGE COMPLEMENT RECEPTOR FUNCTION IN VITRO

IV. The Lymphokine that Activates Macrophage C3 Receptors for Phagocytosis Binds to a Fucose-bearing Glycoprotein on the Macrophage Plasma Membrane

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Work from this laboratory has identified a unique lymphokine that activates macrophage receptors for the third complement component (C3) so that they promote phagocytosis of C3-coated particles (1–6). The lymphokine exerts its effect via a novel mechanism. Macrophage C3 receptors are normally unable to move within the cell's plasma membrane and, even though they promote very efficient particle binding, are unable to promote phagocytosis (7). The lymphokine acts by freeing the immobilized receptors, enabling them to move within the cell's plasma membrane (5). When ligated by particle-bound C3, the mobile receptors are free to move laterally, perhaps to aggregate among themselves or, more likely, to become associated with other plasma membrane or subplasmalemmal molecules that act as second messengers, thereby generating and propagating a phagocytic signal, which activates the cell's intracellular phagocytic machinery.

To better understand the mechanism by which the lymphokine acts, it would be ideal to identify the macrophage molecule(s) with which the mediator interacts. It seemed likely that the lymphokine interacts first at the macrophage surface, perhaps via a specific lymphokine receptor. In the present studies, we sought evidence for such a receptor. The results strongly suggest that the lymphokine binds to a fucose-containing glycoprotein on the macrophage surface.

Materials and Methods

Reagents and Media. Dispase was obtained from Boehringer Mannheim Biochemicals, Indianapolis, IN; L-fucose, D-fucose, mannose, D-galactose, *N*-acetyl-D-glucosamine, glucose, arabinose, 2-deoxy-D-glucose, D-xylose, gorse lectin, wheat germ agglutinin, pokeweed mitogen, phytohemagglutinin (PHA),[1] concanavalin A (Con A), trypsin (lot T8253, twice recrystallized), and fucosidase were obtained from Sigma Chemical Co., St. Louis,

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[1] *Abbreviations used in this paper:* C3, third component of complement; Con A, concanavalin A; E, sheep erythrocytes; EIgG, E coated with anti-E IgG; EIgM, E coated with anti-E IgM; EIgMC, EIgM coated with the first four complement components; FBS, fetal bovine serum; LDCF, lymphocyte-derived chemotactic factor; MIF, migration inhibitory factor; PBS, phosphate-buffered saline; PHA, phytohemagglutinin; PMA, phorbol myristate acetate.

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MO; glutaraldehyde, 50% aqueous solution, was purchased from Fisher Scientific Co., Atlanta, GA; ovomucoid trypsin inhibitor was obtained from Worthington Biochemical Corp., Freehold, NJ; medium 199 was purchased from Microbiological Associates, Walkersville, MD; and fetal bovine serum (FBS) and penicillin-streptomycin were obtained from Gibco Laboratories, Grand Island, NY. FBS was heat inactivated (56°C for 30 min) before use.

Sheep erythrocytes (E) in Alsever's solution, obtained from Scott Laboratories, Fiskeville, RI, were washed three times in phosphate-buffered saline (PBS) and resuspended in medium. A 4.05% aqueous solution of Brewer thioglycollate medium (Difco Laboratories, Detroit, MI) was prepared according to the manufacturer's directions.

Immunologically Coated Sheep E. Rabbit anti-sheep E IgM and rabbit anti-sheep E IgG were obtained from Cordis Laboratories, Miami, FL. Sheep E coated with anti-E IgG (EIgG) or with anti-E IgM and the first four complement components (EIgMC) were prepared as previously described (7).

Animals. Peritoneal macrophages, thymocytes, and serum used to prepare immobilized immune complexes were obtained from 20–30 g female, CD-1 Swiss mice (Charles River Breeding Laboratories, Inc., Wilmington, MA). C5-deficient mouse serum was obtained from female C5-deficient AKR mice (The Jackson Laboratory, Bar Harbor, ME). 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 on glass coverslips in petri dishes, as previously described (8). Macrophages elicited with thioglycollate medium were harvested 4 d after intraperitoneal injection of 1 ml/mouse of thioglycollate medium and cultured in the same manner as resident peritoneal macrophages.

Preparation of Coverslips. Coverslips used for phagocytosis assays were sterilized and used without further treatment. Coverslips used for C3 receptor mobility assays were coated with complement-containing immune complexes as previously described (5, 9).

Preparation of Lymphokine. Culture supernatants containing the lymphokine that enables resident mouse peritoneal macrophages to ingest C3-coated particles were prepared and used as previously described (1, 2).

Assessment of Macrophage Fc and C3 Receptor Function. After a 48-h incubation in medium-10% FBS, macrophage monolayers were washed, some were subjected to the treatments indicated, washed again, some were overlaid with lymphokine-containing supernatant, 0.2 ml of 0.5% EIgG or EIgMC was added to the dishes, and cultures incubated for 30 min 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.

Assessment of Macrophage C3 Receptor Mobility. Freshly harvested mouse peritoneal macrophages were plated on coverslips coated with complement-containing immune complexes. After a 1-h incubation to allow macrophages to adhere, coverslips were washed, some were subjected to the treatments indicated, washed again, and some were overlaid with lymphokine-containing supernatant. After a 15-min incubation at 37°C, cultures were washed and the macrophages' ability to bind EIgMC assessed as previously described (5).

Presentation of Results. Each result reported is the average of at least three separate determinations, each performed in duplicate. Attachment Index and 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 the mean ± SEM.

Results

Effect of Protease Treatment on the Macrophage's Ability to Respond to the Lymphokine. Cell surface receptors for a number of ligands are proteins (10). We

initially sought evidence for a protein receptor for the lymphokine by attempting to destroy it with proteases. Experiments using varying concentrations of trypsin were unsuccessful because, even in fairly low concentrations, trypsin treatment destroyed macrophages' C3 receptors (data not shown), thereby abolishing the assays for lymphokine activity, C3 receptor-mediated particle attachment, and phagocytosis.

Dispase, a neutral protease from *Bacillus polymyxa* (11), proved ideal for these studies because it had no effect on macrophages' C3 receptors. Macrophages were treated with varying concentrations of Dispase at 37°C for 30 min, after which they were washed, overlaid with lymphokine, and assessed for their ability to phagocytize EIgMC. In a dose-dependent fashion, Dispase treatment abolished the macrophage's ability to respond to the lymphokine and phagocytize C3-coated E (Fig. 1). Dispase did not exert its effect by killing or non-specifically injuring macrophages because Dispase-treated macrophages could phagocytize EIgG normally (data not shown) and could recover completely the ability to respond to the lymphokine and phagocytize via their C3 receptors within several hours of removal of the enzyme (Fig. 2).

Because the assay for lymphokine activity was phagocytosis mediated by macrophages' C3 receptors, failure of macrophages to ingest EIgMC could have been due either to Dispase's affecting the C3 receptors per se or to its interfering with the lymphokine's interaction with macrophages. To distinguish between these possibilities, we performed the following control experiments. First, we assessed the ability of Dispase-treated macrophages to bind EIgMC; it was normal and no different from that of nontreated macrophages (data not shown). Second, we assessed the ability of thioglycollate-elicited macrophages, cells that spontaneously phagocytize via their C3 receptors, to ingest EIgMC after Dispase treatment. Dispase had no effect on these cells' ability to phagocytize EIgMC (Fig. 1). Thus, it is very unlikely that Dispase treatment affected macrophage's

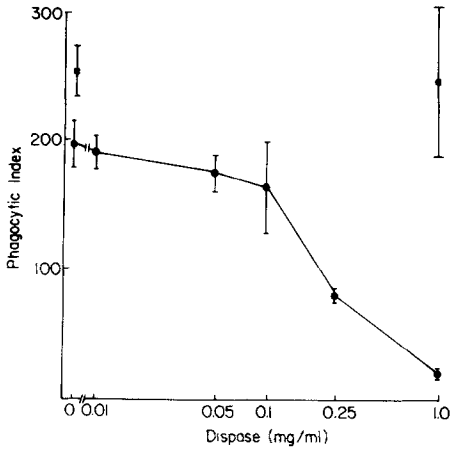

FIGURE 1. Effect of Dispase treatment on macrophages' ability to respond to the lymphokine and to phagocytize EIgMC. Resident (—●—) or thioglycollate-elicited (—■—) mouse peritoneal macrophages were treated with Dispase for 30 min at 37°C. The cells were washed, resident macrophages were overlaid with lymphokine, and both macrophage populations were assayed for their ability to ingest EIgMC.

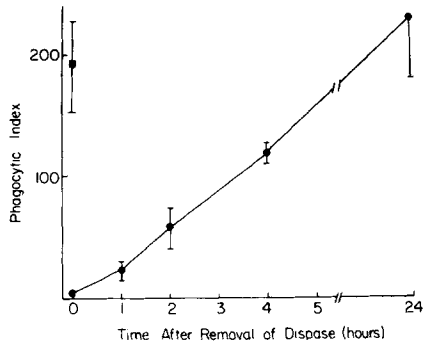

FIGURE 2. Recovery of macrophages from the effects of Dispase treatment. Resident mouse peritoneal macrophages that had been treated with 1 mg/ml of Dispase for 30 min at 37°C were washed and overlaid with medium. At the times indicated these cells were assayed for their ability to ingest EIgMC (—●—). The Phagocytic Index of macrophages that were not subjected to Dispase treatment is indicated (■).

C3 receptors. Rather, its effect was on the interaction of the lymphokine with the macrophage, probably interference with the binding of the lymphokine to a cell surface protein.

We have previously found that there is a precise correlation between mobility of macrophage C3 receptors and the ability of the receptors to promote phagocytosis (5, 6). Moreover, we found that manipulations of macrophages that abrogated one response to the lymphokine, C3 receptor-mediated phagocytosis, also abrogated the other response, C3 receptor mobility (5, 6). To determine whether or not Dispase treatment abrogated both lymphokine effects, we assessed the C3 receptor mobility of Dispase-pretreated, lymphokine-treated macrophages by determining the ability of macrophages plated on complement-containing immune complexes and treated first with Dispase and then with lymphokine to bind EIgMC. In this assay, C3 receptors that are mobile become trapped on the immobilized immune complexes when they diffuse randomly from the nonadherent to the adherent macrophage surface; macrophages with mobile receptors therefore lose the ability to bind EIgMC. In contrast, C3 receptors that are immobile are unable to diffuse within the macrophage plasma membrane and remain in place even when macrophages are plated on immune complexes; these macrophages therefore retain the ability to bind EIgMC. Dispase treatment of macrophages abrogated lymphokine-induced C3 receptor mobility because Dispase-pretreated, lymphokine-treated macrophages retained the ability to bind EIgMC (Table I, line 4).

Effect of Sugars on the Interaction of the Lymphokine with Macrophages. Receptors for a number of ligands are glycoproteins (12–15), and specific sugar residues on receptor molecules have been found to be essential in the binding of certain ligands (16–20). We sought a role for specific sugars in mediating lymphokine binding by trying to competitively inhibit its binding with a number of sugars. Experimental sugars were added at either 0.1 M or other indicated concentrations to lymphokine-containing supernatants. After a 15-min incubation at 37°C, these supernatants, containing both lymphokine and sugar, were added to macrophages and the macrophages' ability to ingest EIgMC assessed.

TABLE I

Effect of Dispase Treatment of Macrophages on Lymphokine-induced C3 Receptor Mobility

Treatment of macrophages with:		Attachment Index of EIgMC
Dispase	Lymphokine	
−	−	1,030 ± 140
+	−	998 ± 184
−	+	41 ± 3
+	+	729 ± 175

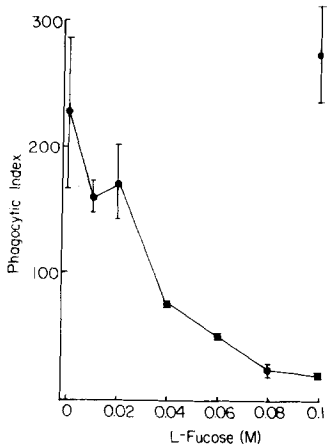

FIGURE 3. Effect of L-fucose on the ability of lymphokine to activate macrophage C3 receptors for phagocytosis. L-Fucose was added in the concentrations indicated to lymphokine-containing supernatants. Resident mouse peritoneal macrophages (—●—) were overlaid with the supernatants and assayed for their ability to ingest EIgMC. The ability of thioglycollate-elicited macrophages to ingest EIgMC in the presence of L-fucose was also assessed (■).

Inclusion of L-fucose in the incubation mixture abolished, in a dose-dependent manner, the ability of macrophages to respond to the lymphokine and phagocytize EIgMC (Fig. 3). L-Fucose did not exert its effect simply by harming the cells because L-fucose–treated macrophages ingested EIgG normally (data not shown). Moreover, L-fucose treatment had no direct effect on macrophages' C3 receptors because L-fucose–treated, thioglycollate-elicited macrophages retained the ability to phagocytize EIgMC (Fig. 3). Finally, L-fucose appeared either to bind to the lymphokine or to otherwise affect the lymphokine-macrophage interaction but not to bind to macrophages, because macrophages pretreated with L-fucose and then washed retained the ability to respond to the lymphokine and phagocytize EIgMC (data not shown).

Of several other pentoses and hexoses tested, including the stereoisomer of L-fucose, D-fucose, none was able to abrogate the lymphokine's effect on macrophages (Table II). In addition, only L-fucose prevented lymphokine-mediated complement receptor mobility (Table III). The most likely mechanism by which L-fucose interfered with the lymphokine's action on macrophages is by competi-

TABLE II
Effects of Sugars on the Ability of Lymphokine to Activate Macrophage C3 Receptors for Phagocytosis

Sugar added at 0.1 M to lymphokine	Phagocytic Index of EIgMC
None	201 ± 50
L-Fucose	37 ± 12
D-Fucose	190 ± 56
Mannose	227 ± 47
D-Galactase	189 ±47
N-Acetyl-D-glucosamine	239 ± 46
Glucose	211 ± 59
2-Deoxy-D-glucose	156 ± 15
Arabinose	141 ± 11
Xylose	154 ± 10

TABLE III
Effects of Sugars on Lymphokine-induced Macrophage C3 Receptor Mobility

Sugar added at 0.1 M to lymphokine	Attachment Index of EIgMC
None	55 ± 8
L-Fucose	955 ± 22
D-Fucose	51 ± 1
Mannose	157 ± 120
D-Galactase	39 ± 11
N-Acetyl-D-glucosamine	42 ± 15
Glucose	64 ± 8
2-Deoxy-D-glucose	128 ± 49
Arabinose	69 ± 24
Xylose	63 ± 17

tively binding the lymphokine and preventing its binding to L-fucose residues on the macrophage surface.

Effect of Abolishing and Masking Macrophage Surface Fucose Residues on the Macrophage's Ability to Respond to the Lymphokine. We explored further the requirement for cell surface fucose in two additional ways, by removing fucose residues with the enzyme fucosidase and by masking fucose residues with a fucose-specific lectin, gorse lectin.

In the first experiments, macrophages were treated with 0.0017 U/ml of fucosidase for 1 h at 37°C. Cells were washed to remove the enzyme and overlaid with lymphokine, and their ability to phagocytize EIgMC was determined. These cells were unable to phagocytize EIgMC (Fig. 4). Within a few hours after removal of the enzyme, they were fully able to respond to the lymphokine (Fig. 4), indicating that fucosidase did not exert its effect by being cytotoxic. Control experiments revealed that fucosidase treatment had no effect on macrophages' ability to bind EIgMC or on the ability of thioglycollate-elicited macrophages to phagocytize EIgMC (data not shown). Parallel experiments revealed that the C3

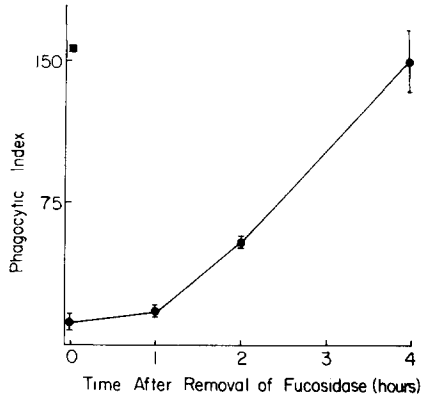

FIGURE 4. Effect of fucosidase treatment on macrophages' ability to respond to the lymphokine. Macrophages were treated with 0.0017 U/ml of fucosidase for 30 min at 37°C, washed, and assayed for their ability to ingest EIgMC immediately and at varying times after removal of fucosidase (—●—). The ability of macrophages that were not treated with fucosidase to ingest EIgMC was also assessed (■).

TABLE IV

Effect of Fucosidase Treatment of Macrophages on Lymphokine-induced C3 Receptor Mobility

Treatment of macrophages with:		Attachment Index of EIgMC
Fucosidase	Lymphokine	
−	−	1,047 ± 138
+	−	1,036 ± 185
−	+	53 ± 5
+	+	1,002 ± 184

receptors of fucosidase-pretreated macrophages remained immobile even after lymphokine treatment (Table IV, line 4).

In other experiments, macrophages were treated with 5 μg/ml of a lectin for 30 min at 37°C. Non-bound lectin was removed by washing, cells were overlaid with lymphokine, and their ability to ingest EIgMC was assessed. Macrophages that had been pretreated with gorse lectin, which binds specifically to fucose (21), were unable to ingest EIgMC (Table V, line 2). Gorse lectin did not act simply by perturbing the macrophage surface or by cross-linking or aggregating cell surface components because treatment of macrophages with any of five other lectins, none of which binds fucose, was without effect (Table V). Control experiments revealed that gorse lectin treatment had no effect on the ability of macrophages to bind EIgMC or on the ability of thioglycollate-elicited macrophages to ingest EIgMC (data not shown).

In parallel experiments, treatment of macrophages with gorse lectin, which prevented lymphokine activation of the cells' C3 receptors for phagocytosis, also prevented the lymphokine from mobilizing the macrophages' C3 receptors (Table VI, line 2). Contrariwise, treatment of macrophages with lectins that did not alter one effect of the lymphokine on macrophages failed to alter the other lymphokine effect (Table VI), except in the case of Con A. Con A–treated

TABLE V
Effect of Lectin Treatment on Macrophages' Ability to Respond to the Lymphokine

Lectin treatment	Sugar specificity of lectin	Phagocytic Index
None		205 ± 29
Gorse	Fucose	16 ± 12
Lentil	Mannose/Glucose	172 ± 8
Wheat germ	*N*-acetyl-D-glucosamine	164 ± 21
Pokeweed	?	208 ± 52
PHA	?	291 ± 101
Con A	Mannose	197 ± 40

TABLE VI
Effect of Lectin Treatment of Macrophages on Lymphokine-induced C3 Receptor Mobility

Lectin treatment	Attachment Index of EIgMC by macrophages	
	Nontreated	Lymphokine-treated
None	1,041 ± 130	65 ± 10
Gorse	950 ± 80	869 ± 96
Lentil	1,183 ± 64	89 ±14
Wheat germ	1,166 ± 87	99 ± 4
Pokeweed	1,078 ± 89	69 ±16
PHA	1,061 ± 103	83 ± 29
Con A	817 ± 56	698 ± 128

macrophages retained the ability to respond to the lymphokine and phagocytize EIgMC; however, these macrophages also retained the ability to bind EIgMC even when plated on complement-containing immune complexes and treated with lymphokine (Table VI, line 7), suggesting that their C3 receptors were immobile yet able to promote phagocytosis. If so, our hypothesis that only mobile receptors are capable of promoting phagocytosis would be open to serious question.

We considered the possibility that the C3 receptors of macrophages plated on C3-containing immobilized immune complexes and treated first with Con A and then with lymphokine may have been mobile and may have become sequestered on the adherent macrophage surface but that these macrophages retained the ability to bind EIgMC not via their C3 receptors but rather by macrophage-bound Con A. This possibility seemed plausible because we have previously used Con A to link E to macrophages (2). If this were the mechanism by which Con A–pretreated, lymphokine-treated macrophages bound EIgMC, then these macrophages should be able to bind E and EIgM as well as EIgMC, and inclusion of mannose in the incubation mixture containing macrophages and EIgMC should markedly reduce EIgMC binding. As shown in Table VII, these are precisely the results we obtained. Thus, binding of EIgMC by these cells was mediated by macrophage-bound Con A. The cells' C3 receptors were mobile and were sequestered on the adherent macrophage surface. Therefore, none of the control

TABLE VII

Effect of Con A Treatment on Macrophages' Interaction with Sheep Erythrocytes

Addition to incubation mixture	Particle offered	Attachment Index by Con A-treated macrophages
—	EIgMC	571 ± 74
—	E*	151 ± 19
—	EIgM*	214 ± 18
Mannose	EIgMC	123 ± 37

* Attachment indices for E and EIgM by macrophages that had not been treated with Con A were 7 and 6, respectively.

lectins influenced either lymphokine activation of C3 receptor-mediated phagocytosis or lymphokine-induced C3 receptor mobility.

Results of these experiments, using fucosidase and using gorse lectin and other lectins, indicate that macrophage surface fucose is essential for macrophages to respond to the lymphokine that activates their C3 receptors for phagocytosis. It is most likely that fucose constitutes a portion of the lymphokine-binding site, probably in a glycoprotein lymphokine receptor.

Discussion

Many molecules that elicit responses from cells act by binding to specific receptors on the cell's plasma membrane. Ligation of a receptor by the ligand modifies the receptor in such a way that it acquires new properties, often the ability to interact with second messenger molecules, which can then interact with other intracellular molecules (22). In this way ligand binding initiates signals that are transduced into activation or suppression of cellular processes located at sites distant from the ligand and the receptor (22).

In the present studies we tested the hypothesis that the lymphokine that activates macrophage C3 receptors for phagocytosis exerts its effect by first binding to a receptor on the macrophage surface. The results obtained indicate that the lymphokine must bind to a fucose-containing glycoprotein on the macrophage surface, perhaps a specific receptor molecule. The protein nature of the molecule was deduced by the inability of Dispase-treated macrophages to respond to the lymphokine, whereas the requirement for fucose was demonstrated by competitive inhibition, by cleaving fucose from the macrophage surface, and by masking cell surface fucose with a lectin.

Human (23) and guinea pig migration inhibitory factor (MIF) (24) and human lymphocyte-derived chemotactic factor (LDCF) (25) appear to bind to a fucose-bearing macrophage surface structure, as evidenced by competitive inhibition studies and by treatment of cells with fucosidase. No data were presented in those studies to indicate whether or not the lymphokine-binding molecule was a protein. The similarity of binding requirements for different lymphokines raises several possibilities regarding the structure of the lymphokines and the nature of the lymphokine receptor(s).

MIF, LDCF, and the lymphokine that activates macrophage complement receptors might be the same molecule. However, because the latter lymphokine

is not generated by mechanisms that generate the first two (1), because its apparent molecular weight (~10,000) is lower than that of MIF (2), and because lymphokines from three species as divergent as man, mouse, and guinea pig are unlikely to be identical molecules, this possibility seems remote. However, it is possible that one of the lymphokines might be derived, e.g., by cleavage, from another or that the lymphokines have similar or identical binding sites. If the second possibility is correct, then the lymphokines may be a family of molecules derived originally by gene duplication and preserved over a long evolutionary period.

There is very little information concerning the nature of putative lymphokine receptor sites. The present studies suggest that the site to which the lymphokine that activates macrophage C3 receptors binds is a glycoprotein, and this and other studies (23–25) demonstrate the critical role of fucose residues in mediating binding of a number of lymphokines. These results suggest that either a single receptor may recognize a number of different lymphokines or that different lymphokine receptors bear structural similarities, especially in their ligand-binding sites. These distinctions can be made only as the receptors are purified and studied at the molecular level. Gorse lectin, which binds the lymphokine that activates macrophage C3 receptors and presumably would bind to the receptor(s) for MIF and LDCF as well, might prove very useful in purification of lymphokine receptors from macrophage plasma membranes.

Triggering receptors with lectins often induces a cellular response that mimics the response induced by the natural ligand. Examples include mitogenesis of lymphocytes induced by Con A or PHA (26) and the induction of chemotaxis of human neutrophils by the fucose-binding *Lotus tetragonolobus* lectin (27). In the present studies, gorse lectin presumably bound to the lymphokine receptor and prevented lymphokine binding but did not itself induce the lymphokine effect. Thus, the lectin acted as a competitive antagonist, indicating that the manner in which a lymphokine receptor is ligated, rather than simply ligation per se, determines whether or not the bound ligand will activate the receptor to produce a biologic response.

We have previously presented evidence that the lymphokine activates macrophage C3 receptors for phagocytosis by freeing anchored receptors and allowing them to diffuse within the cell's plasma membrane (5, 6). In the present experiments, whenever a treatment of macrophages or of lymphokine prevented lymphokine activation of macrophage C3 receptors for phagocytosis, that treatment also prevented lymphokine-induced C3 receptor mobility. These results strengthen our hypothesis that, for a receptor to be able to promote phagocytosis, it must be able to diffuse within the macrophage plasma membrane.

In conjunction with previous studies of the lymphokine that activates macrophage C3 receptors (1–6), the present studies represent an example in which one receptor affects the function of another receptor by inducing a qualitative change in receptor behavior. Other ligand-receptor interactions have been shown to induce qualitative changes in C3 receptor behavior in other cells. For example, binding of fibronectin to fibronectin receptors or of phorbol myristate acetate (PMA) to PMA receptors of human monocytes activated the cells' C3b receptors and C3bi receptors for phagocytosis (28–30). In other studies, ligating human

monocytes' C3b receptors induced changes not only in the distribution of C3b receptors but also in the topography of the cells' Fc receptors, and, conversely, ligating Fc receptors altered the topography of both Fc and C3b receptors (31). Similarly, ligating chemotactic factor receptors of neutrophils caused previously randomly distributed Fc receptors to accumulate at the cell's leading edge (32). Finally, ligating the human neutrophil C3bi receptor, or a closely associated plasma membrane structure, with antibody abrogated the ability of the cell's Fc receptors and C3b receptors to promote phagocytosis, without affecting their ability to bind ligand-coated particles (33), strongly suggesting that the C3bi receptor normally regulates the function of both C3b and Fc receptors. Further evidence in support of that hypothesis is the finding that neutrophils which, as a consequence of a genetic defect, lack the C3bi receptor, or a closely related protein, are unable to phagocytize via either their C3b receptors or their Fc receptors (34). Further studies of the interactions between these immunologic receptors may provide valuable insights into general mechanisms by which receptors communicate with one another and by which receptors regulate functions not only of intracellular molecules but also of cell surface structures.

Summary

Macrophage receptors for the third component of complement (C3) are normally immobilized and unable to diffuse within the cell's plasma membrane and, even though they promote avid particle binding, are unable to promote phagocytosis of C3-coated particles. We have previously identified a lymphokine that activates macrophage C3 receptors for phagocytosis and have found that it acts by freeing the receptors so that they can diffuse within the macrophage plasma membrane.

It seemed likely to us that the initial lymphokine-macrophage interaction would occur at the macrophage surface, perhaps via a specific lymphokine receptor. Since the binding of many ligands to cells is mediated by cell surface glycoproteins, we examined the protein and sugar requirements for murine peritoneal macrophages to respond to the lymphokine. Macrophages treated with the neutral protease Dispase lost the ability to respond to the lymphokine, and inclusion of L-fucose in the incubation medium containing lymphokine and macrophages inhibited markedly the macrophages' response to the lymphokine, suggesting that the lymphokine exerts its effects by first binding to fucose residues on a glycoprotein receptor on the macrophage surface. Further evidence for the essential role of macrophage surface fucose was obtained by demonstrating that pretreatment of macrophages with either fucosidase or gorse lectin, a fucose-binding lectin, strikingly disabled the cells from responding to the lymphokine. All treatments that prevented lymphokine activation of macrophage C3 receptors for phagocytosis also prevented lymphokine-induced C3 receptor mobility.

These results strongly suggest that the lymphokine binds to a fucose-bearing macrophage surface glycoprotein, perhaps a specific lymphokine receptor. They also strengthen our hypothesis that, for a receptor to be able to promote phagocytosis, it must be able to diffuse within the macrophage plasma membrane.

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