Contribution of mannose receptor to signal transduction in Fcγ receptor–mediated phagocytosis of mouse peritoneal macrophages induced by liposomes

Makoto Murai, Yukihiko Aramaki, and Seishi Tsuchiya
Tokyo College of Pharmacy, Tokyo, Japan

Abstract: The contribution of mannose receptors on the cell surface of mouse peritoneal macrophages to the process of liposome-induced phagocytosis of immunoglobulin G-opsonized sheep red blood cells (SRBCs) through Fcγ receptor has been investigated. Fcγ receptor-mediated phagocytosis of opsonized SRBCs was activated by modified α_2-macroglobulin, which was produced in the incubation mixture of α_2-macroglobulin and liposome-treated splenic B cells. The phagocytosis was specifically inhibited by the addition of D-mannose, and the inhibition was dependent on the D-mannose concentration. The binding of modified α_2-macroglobulin to macrophages was also reduced by the addition of D-mannose. The activation effect of modified α_2-macroglobulin was not inhibited when in the presence of α_2-macroglobulin-trypsin and -methylamine complexes. In the presence of cycloheximide, activated phagocytosis was reduced to the control level. By Scatchard plot analysis of IgG binding studies, the number of Fcγ receptors of a macrophage had been increased to 4.6-fold that of a control macrophage by treatment with modified α_2-macroglobulin. These findings suggest that macrophage mannose receptors are involved in activating the process of Fcγ receptor-mediated phagocytosis of opsonized SRBCs induced by modified α_2-macroglobulin. Lectins may participate in a signal transduction in macrophage activation by liposomes. *J. Leukoc. Biol.* 57: 687–691; 1995.

Key Words: *Fcγ receptor · lectin · mannose receptor · liposome · α_2-macroglobulin · macrophage*

INTRODUCTION

In mammalian tissues, several lectins have been described with different sugar specificities, such as hepatic lectins for galactose residues [1–3] and macrophage receptors for mannose and galactose residues [1, 4–6]. It has been shown that galactose-specific lectins of hepatocytes or macrophages recognize the desialylated sugar structure of catabolites of serum glycoprotein or aged erythrocytes, resulting in rapid sequestration from the blood stream [4, 5, 7, 8]. However, the biological role of lectin is not fully understood.

We reported that liposomes consisting of dipalmitoylphosphatidylcholine, hydrogenated phosphatidylserine, and cholesterol also activated mouse peritoneal macrophages and enhanced Fcγ receptor-mediated ingestion of immunoglobulin G (IgG)-opsonized sheep red blood cells (SRBCs) [9, 10]. During this activation process, liposomes did not activate macrophages directly, but serum and B cells were required, and α_2-macroglobulin was identified as a macrophage-activating serum factor. Mannose residue(s) at the terminal of the sugar chain of this glycoprotein modified by liposome-treated B cells is expected to be a key ligand for macrophage activation (M. Murai, Y. Aramaki, S. Tsuchiya, submitted).

The purpose of this work is to clarify the contribution of mannose receptors to the Fcγ receptor-mediated phagocytosis of IgG-opsonized SRBCs by mouse peritoneal macrophages. With the addition of D-mannose, binding of modified α_2-macroglobulin to macrophages and ingestion of opsonized SRBCs were inhibited, and the increase in the number of Fcγ receptors induced by modified α_2-macroglobulin was also reduced to control levels. Receptors having specificity for mannose residues thus may contribute to the enhancement of Fcγ receptor-mediated phagocytosis of mouse peritoneal macrophages induced by liposome treatment.

MATERIALS AND METHODS

Animals

Inbred BALB/c (8–10 weeks) male mice were purchased from Japan SLC (Shizuoka, Japan).

Liposome preparation

Multilamellar liposomes of dipalmitoylphosphatidylcholine (Nippon Oil and Fat Co., Tokyo, Japan), phosphatidylserine (Nisshin Seiyu Co., Tokyo, Japan), and cholesterol (Wako Pure Chem. Ind., Osaka, Japan) 3.2:2:4.8 (molar ratio) were prepared by the vortexing method as described previously [10].

Cell preparation

Peritoneal exudate cells from BALB/c mice were prepared according to the procedure described previously [10]. Peritoneal exudate cells (1×10^6) suspended in 1 ml of RPMI 1640 medium containing 0.1% bovine serum albumin (BSA medium) were overlaid on a circular glass coverslip (15 mm) that had been placed in a well (22 mm) of a tissue culture plate (Corning Glass Works). The plate was held at 37°C in a humidified 5% CO_2 incubator for 30 min to allow macro-

Abbreviations: BSA, bovine serum albumin; DBP, vitamin D-binding protein; HRP, horseradish peroxidase; IgG, immunoglobulin G; PBS, phosphate-buffered saline; SRBC, sheep red blood cell.

Reprint requests: Y. Aramaki, Tokyo College of Pharmacy, 1432-1 Horinouchi, Hachioji, Tokyo 192-03, Japan.

Received October 25, 1994; accepted December 9, 1994.

phage adherence. Each coverslip was washed with gentle agitation with warmed RPMI 1640 to dislodge nonadherent cells, and a macrophage monolayer was obtained.

Splenic lymphoid cells from BALB/c mice were collected and processed as described by Hashimoto et al. [11]. The B cell population was isolated by removing T cells from the splenic nonadherent cells via treatment with anti-Thy 1.2 monoclonal antibody and guinea pig complement (Cedarlane Lab., Ontario, Canada) according to the method reported by Suzuki et al. [12].

Treatment of B cells with liposomes and preparation of modified α_2-macroglobulin

Splenic B cells (1×10^6 cells/well) were incubated with liposomes (268 nmol lipid/well) in BSA medium for 30 min as described previously [13]. After being washed, the treated cells (1×10^6 cells/ml) were incubated in serum-free RPMI 1640 medium containing 200 ng/ml commercially available bovine α_2-macroglobulin (Mannheim Yamanouchi, Tokyo, Japan) for 3 h, and the supernatant was obtained by centrifugation (600*g*, 10 min) and designated as modified α_2-macroglobulin. Culture medium, which was obtained by incubation of saline-treated splenic B cells with RPMI 1640 medium containing 200 ng/ml α_2-macroglobulin, was used as control.

Opsonization and ingestion assay

The extent of Fcγ receptor-mediated phagocytosis of opsonized SRBCs (Japan Bio Test, Tokyo, Japan) by peritoneal macrophages was determined according to the method of Bianco et al. [13]. Four milliliters of 5% SRBCs suspended in serum-free RPMI 1640 medium was opsonized by the subagglutinating dilution of IgG (about 40 μg protein/ml). One milliliter of 1% suspension of rabbit anti-SRBC IgG (Inter Cell Technologies, Hopewell, NJ)-opsonized SRBCs was overlaid on a macrophage monolayer following the treatment with modified α_2-macroglobulin for 2 h at 37°C (1×10^6 cells/ml) in a humidified 5% CO_2 incubator for 60 min. Noningested SRBCs were lysed by immersing the coverslips in hypotonic phosphate-buffered saline (PBS) (1:10 dilution) for 10 s. The macrophages were fixed with MeOH, dried, and Giemsa stained. Two hundred macrophages on each coverslip were counted at random and the ingestion index was expressed as the product of the percentage of macrophages that ingested SRBCs and the average number of SRBCs ingested by a macrophage.

Modified α_2-macroglobulin binding to macrophages

Binding of modified α_2-macroglobulin to macrophages was determined by enzyme-linked immunosolubent assay. Macrophages (1×10^6 cells) in each well of a microplate (24 well, Corning Glass Works) were incubated with modified α_2-macroglobulin (10 ng) in the presence of 0–30 mM D-mannose at 4°C for 2 h. After being washed three times with 10 mM PBS containing 0.05% Tween 20 (PBS-Tween), the wells were coated with 1 ml of 3% skim milk in PBS-Tween for 1 h at room temperature to block the nonspecific adsorption of antibodies to the wells. After washing with PBS-Tween, 500 ng of rabbit anti-bovine α_2-macroglobulin monoclonal antibody (IgG class, Yagai Research Center, Yamagata, Japan) was added to each well. After incubation of 4°C for 2 h and washing, horseradish peroxidase (HRP)-conjugated goat IgG having specificity for the Fc region of rabbit IgG (Organon Teknica Co., West Chester, PA) diluted with PBS-Tween (1 μg/ml) was added (0.5 ml/well). Following incubation at 4°C for 2 h and washing, 0.5 ml of 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (150 μg/ml) in 0.1 M citrate buffer (pH 4.0) containing 0.015% H_2O_2 was added. After incubation at 37°C for 20 min, absorbance at 405 nm was measured with a microplate reader (Corona, MTP-32, Tokyo, Japan).

Preparation of α_2-macroglobulin complexes

α_2-macroglobulin-trypsin or -methylamine complex was prepared as described previously (M. Murai et al., submitted). Nondenaturing polyacrylamide gel electrophoresis revealed that these α_2-macroglobulin complexes were fast form (M. Murai et al., submitted).

Analysis of Fcγ receptors

The binding parameter of Fcγ receptor of macrophages was determined according to the modified method of Yagawa et al. [14]. Peritoneal macrophages (1×10^5 cells) were incubated with modified α_2-macroglobulin in the presence or absence of 30 mM D-mannose at 37°C for 2 h. The macrophages washed with PBS-Tween were scraped with a rubber policeman from the culture plate and transferred into a skim milk-coated test tube (Corning Glass Works). Various concentrations of HRP-conjugated mouse IgG (Organon Teknika Co.) or its $F(ab')_2$ fragment (Rockland, Gilbertsville, PA) in BSA medium were added to each tube and incubated at 4°C for 5 h. After washing three times with PBS-Tween, 0.3 ml of substrate solution was added and incubated at 37°C for 20 min. The amount of bound IgG or $F(ab')_2$ fragment was determined by HRP activity measured by the same procedure as for the binding of modified α_2-macroglobulin to macrophages by using the relation that the enzyme activity of 1 ng of HRP-conjugated mouse IgG showed an absorbance of 0.107 (37°C, 20 min). The binding capacity of the Fcγ receptor of macrophages was estimated by subtraction of binding of $F(ab')_2$ from that of IgG. A control experiment was run by the same procedure using control medium instead of modified α_2-macroglobulin.

RESULTS

Effects of monosaccharides on ingestion index

As shown in **Figure 1A,** an ingestion index for IgG-opsonized SRBCs of macrophages, incubated with modified α_2-macroglobulin, was accelerated about 3.5-fold over that of macrophages, incubated with control medium. The ingestion for nonopsonized SRBCs was almost the same as that of macrophages incubated with control medium.

Previously, we reported that modified α_2-macroglobulin contains mannose residue as the terminal sugar chain (M. Murai et al., submitted). So the contribution of mannose receptors to the phagocytosis of opsonized SRBCs via Fcγ receptors was expected, and the effects of monosaccharides on the phagocytosis were investigated. As shown in Figure 1A, a reduction of the ingestion index was observed only when D-mannose was added to the medium containing modified α_2-macroglobulin. The inhibitory effect increased with the concentration, and in the presence of 30 mM D-mannose the ingestion index decreased to the control level (Fig. 1B). Moreover, mannose exposure of BSA or fetuin did not cause macrophage activation (Fig. 1A).

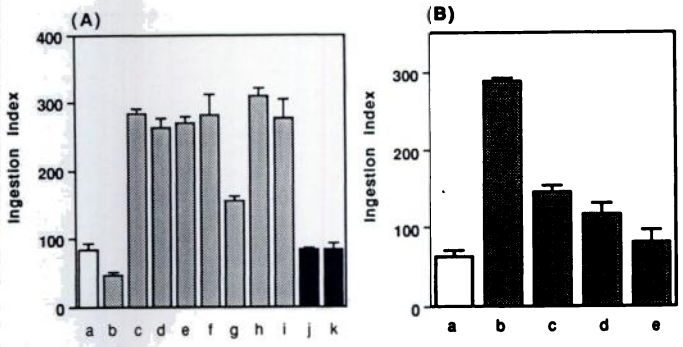

Fig. 1. Effects of various monosaccharides on the ingestion index of opsonized SRBCs by mouse peritoneal macrophages. (A) (a) Control medium prepared by incubation of α_2-macroglobulin with saline-treated B cells; (b and c) modified α_2-macroglobulin prepared by incubation α_2-macroglobulin with liposome-treated B cells; (d) modified α_2-macroglobulin + D-galactose; (e) modified α_2-macroglobulin + D-glucose; (f) modified α_2-macroglobulin + D-fucose; (g) modified α_2-macroglobulin + D-mannose; (h) modified α_2-macroglobulin + *N*-acetyl-β-D-galactosamine; (i) modified α_2-macroglobulin + *N*-acetyl-β-D-glucosamine; (j) mannose-exposed BSA; (k) mannose-exposed fetuin. Except for (b), IgG-opsonized SRBCs were ingested by macrophages. Nonopsonized SRBCs were ingested in (b). Each monosaccharide concentration was 20 mM. Mannose-exposed proteins were prepared using commercially available neuraminidase, β-galactosidase, and β-*N*-acetylglucosaminidase and purified with a concanavalin A-Sepharose 4B column as described previously (M. Murai et al., submitted). (B) (a) Control medium; (b) modified α_2-macroglobulin; (c) modified α_2-macroglobulin + D-mannose (10 mM); (d) modified α_2-macroglobulin + D-mannose (20 mM); (e) modified α_2-macroglobulin + D-mannose (30 mM). The data shown are means ± SD of three separate experiments. (□) Macrophages incubated with control medium; (▩) macrophages incubated with modified α_2-macroglobulin.

Binding of modified α_2-macroglobulin to macrophages

Significant binding of modified α_2-macroglobulin to macrophages was observed and this binding decreased with increasing D-mannose concentrations, suggesting that the binding of modified α_2-macroglobulin to macrophages was inhibited by D-mannose (**Fig. 2**). However, no inhibitory effects of other monosaccharides, such as *N*-acetyl-β-D-galactosamine, D-galactose, and D-glucose, were observed (data not shown).

Effect of α_2-macroglobulin complex on ingestion index

α_2-Macroglobulin binds to proteases and small primary amines, such as methylamine, and the complex is immediately trapped by α_2-macroglobulin receptors on macrophages [15, 16]. The contribution of α_2-macroglobulin receptor to macrophage activation induced by modified α_2-macroglobulin was examined using α_2-macroglobulin-trypsin and α_2-macroglobulin-methylamine complexes. These complexes themselves did not affect the ingestion index of macrophages. Also, the accelerated ingestion index of macrophages induced by modified α_2-macroglobulin was not reduced in the presence of 100-fold concentrations (20 μg/ml) of α_2-macroglobulin-trypsin or α_2-macroglobulin-methylamine complex (**Fig. 3**).

Effect of cycloheximide on ingestion index

The accelerated ingestion index of macrophages observed following incubation with modified α_2-macroglobulin was reduced to control levels by the addition of 10 μg/ml cycloheximide (**Fig. 4**).

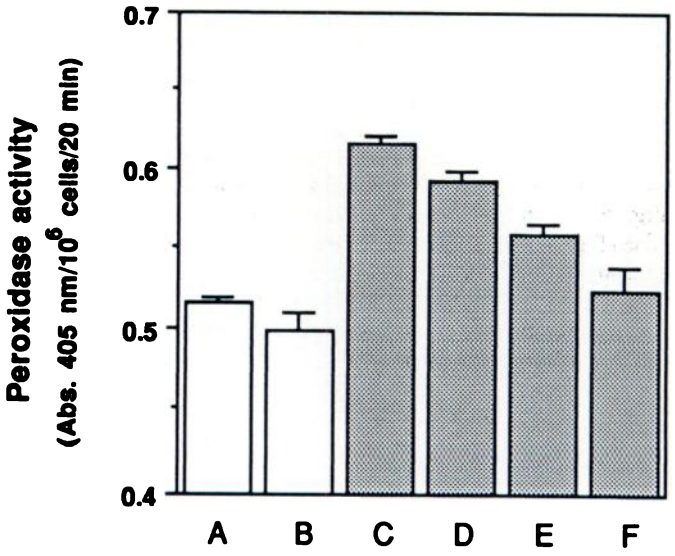

Fig. 2. Binding of modified α_2-macroglobulin to peritoneal macrophages. (A) Control medium; (B) control medium + D-mannose (30 mM); (C) modified α_2-macroglobulin; (D) modified α_2-macroglobulin + D-mannose (10 mM); (E) modified α_2-macroglobulin + D-mannose (20 mM); (F) modified α_2-macroglobulin + D-mannose (30 mM). The data shown are means ± SD of three separate experiments. (□) Macrophages incubated with control medium; (▩) macrophages incubated with modified α_2-macroglobulin.

Binding parameter of Fcγ receptor

The binding parameters of Fcγ receptors were evaluated by the binding of HRP-conjugated mouse IgG to macrophages. IgG binding to macrophages increased with increasing IgG, and a higher binding capacity was observed in macrophages treated with modified α_2-macroglobulin than in those treated with control medium. With the addition of 30 mM

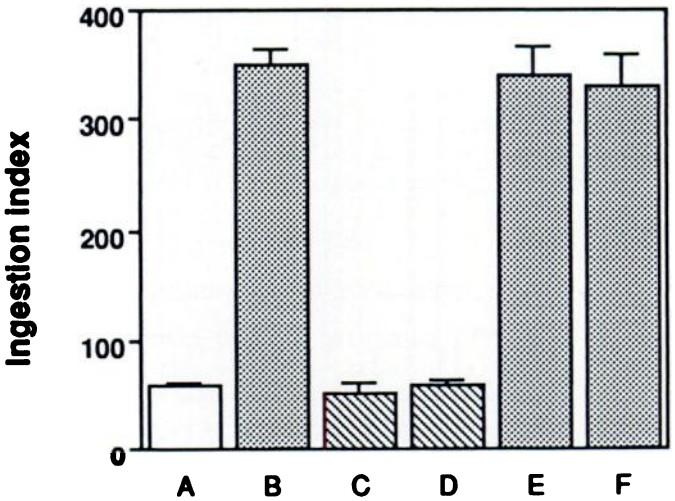

Fig. 3. Effect of α_2-macroglobulin complex on the ingestion index of opsonized SRBCs by mouse peritoneal macrophages treated with modified α_2-macroglobulin. (A) Control medium; (B) modified α_2-macroglobulin (0.2 μg/ml); (C) α_2-macroglobulin-trypsin complex (20 μg/ml); (D) α_2-macroglobulin-trypsin complex (20 μg/ml); (E) modified α_2-macroglobulin (0.2 μg/ml) + α_2-macroglobulin-trypsin complex (20 μg/ml); (F) modified α_2-macroglobulin (0.2 μg/ml) + α_2-macroglobulin-methylamine complex (20 μg/ml). Modified α_2-macroglobulin and α_2-macroglobulin complexes were added to macrophages simultaneously. The data shown are means ± SD of three separate experiments. (□) Macrophages incubated with control medium; (▨) incubated with modified α_2-macroglobulin; (▧) incubated with α_2-macroglobulin complex.

D-mannose, this binding decreased to control levels (**Fig. 5**, inset). The binding of $F(ab')_2$ fragment lacking the Fc portion of IgG was negligible. In addition, the binding of HRP-conjugated IgG to activated macrophages was almost completely inhibited by the addition of 100-fold free mouse IgG. By Scatchard plot analysis, the number of Fcγ receptors of activated macrophages was estimated to be 5.5×10^5 per cell (Fig. 5). This value was 4.6 times higher than the control value (1.2×10^5 per cell). In the presence of D-mannose, the binding was reduced to control levels and the number of Fcγ receptors decreased to 1.8×10^5 per cell. On the other hand, the binding constant was almost equivalent in macrophages treated with control medium (1.5×10^8 M^{-1}), modified α_2-macroglobulin (1.2×10^8 M^{-1}), or modified α_2-macroglobulin in the presence of D-mannose (1.3×10^8 M^{-1}).

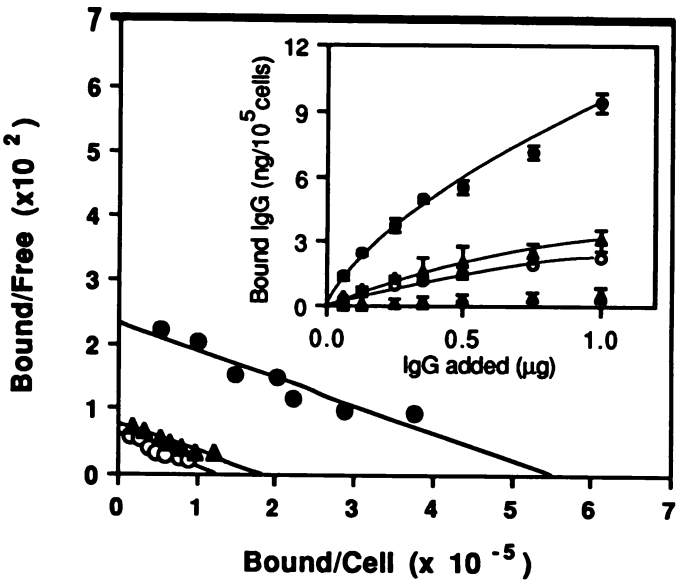

Fig. 5. Scatchard plots of the binding of IgG to macrophages. Macrophages treated with control medium (▲), modified α_2-macroglobulin (●), and modified α_2-macroglobulin + 30 mM D-mannose (○) were incubated with HRP-conjugated IgG. By Schatchard plot analysis, the binding constants for control medium-, modified α_2-macroglobulin-, and modified α_2-macroglobulin + D-mannose-treated macrophages were 1.5×10^8, 1.2×10^8, and 1.3×10^8 (M^{-1}), respectively. The number of binding sites for control medium-, modified α_2-macroglobulin-, and modified α_2-macroglobulin + D-mannose-treated macrophages were 1.2×10^5, 1.8×10^5, and 5.5×10^5 ($cell^{-1}$), respectively. The inset shows a direct plot of the binding of macrophages. Open triangles represent the binding of $F(ab')_2$ to macrophages treated with modified α_2-macroglobulin. The data shown are means ± SD (direct plot) or means (Schatchard plot) of three separate experiments.

DISCUSSION

We have previously reported the effect of liposomes on various functions of mouse peritoneal macrophages [9]. In particular, Fcγ receptor-mediated phagocytosis of opsonized SRBCs by macrophages was strongly enhanced, and this macrophage activation was induced by modified α_2-macroglobulin, which was prepared by incubation of α_2-macroglobulin and liposome-treated B cells. Since the mannose residue at the terminal of the sugar chain of modified α_2-macroglobulin was essential for macrophage activation (M. Murai et al., submitted) a contribution of mannose receptors on macrophages is expected.

To clarify the contribution of mannose receptors to phagocytosis of opsonized SRBCs via Fcγ receptors, the effects of monosaccharides on modified α_2-macroglobulin-induced phagocytosis were investigated. The ingestion index of macrophages enhanced by modified α_2-macroglobulin was specifically reduced by D-mannose (Fig. 1). This result suggested that binding of modified α_2-macroglobulin to mannose receptors of macrophages plays a key role in the enhancement of Fcγ receptor-mediated ingestion of opsonized SRBCs. The activity of HRP bound to the secondary antibody, which was presumed to be the binding of α_2-macroglobulin, was only inhibited by the addition of mannose in a dose-dependent manner (Fig. 2). These results indicate that modified α_2-macroglobulin binds to macrophages through a receptor for mannose. Binding of native α_2-macroglobulin, which has no macrophage-activating capacity, to macrophages was also observed, but this binding was not inhibited by the addition of D-mannose. Thus, the native α_2-macroglobulin may bind to macrophages with nonspecific interactions.

α_2-Macroglobulin can bind with various proteases, and the protease-trapped α_2-macroglobulin mediates several cellular responses following their recognition by α_2-macroglobulin receptor [17, 18]. However, the complex of α_2-macroglobulin with trypsin or methylamine did not activate macrophages (Fig. 3). The present report should rule out a possible contribution of α_2-macroglobulin receptors to macrophage activation induced by modified α_2-macroglobulin, because their activation effect was not reduced in the presence of competitor for α_2-macroglobulin receptor, α_2-macroglobulin-trypsin or -methylamine complex (Fig. 3).

To explain the detailed mechanism of the activation of Fcγ receptor-mediated phagocytosis after the binding of modified α_2-macroglobulin and mannose receptor, two pos-

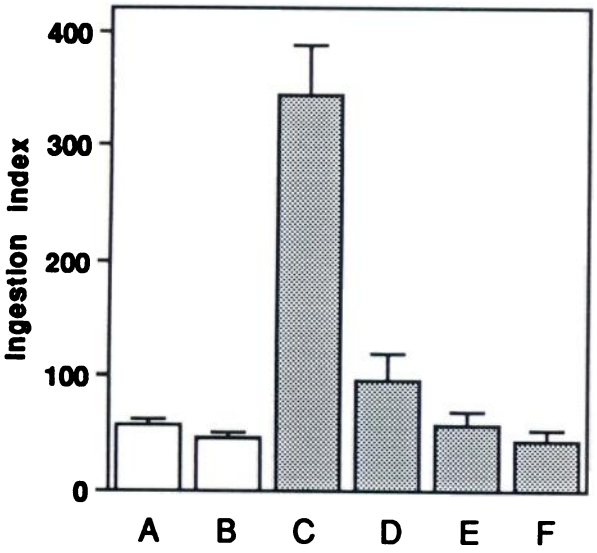

Fig. 4. Effect of cycloheximide on the ingestion index of opsonized SRBCs by mouse peritoneal macrophages treated with modified α_2-macroglobulin. (A) Control medium; (B) control medium + cycloheximide (50 μg/ml); (C) modified α_2-macroglobulin; (D) modified α_2-macroglobulin + cycloheximide (5 μg/ml); (E) modified α_2-macroglobulin + cycloheximide (10 μg/ml); (F) modified α_2-macroglobulin + cycloheximide (50 μg/ml). As for (B), (D), (E), and (F), following the pretreatment of macrophages with cycloheximide for 30 min, control medium or modified α_2-macroglobulin containing each concentration of cycloheximide was added to the macrophages. The data shown are means ± SD of three separate experiments. (□) Macrophages incubated with control medium; (▩) macrophages incubated with modified α_2-macroglobulin.

sibilities should be considered: (1) an increase in the number of Fcγ receptors and (2) an enhancement of the function of Fcγ receptors. Macrophage activation induced by modified α_2-macroglobulin was completely reduced by pretreatment with cycloheximide, a protein synthesis inhibitor (Fig. 4). This result suggests that protein synthesis is involved in this process; that is, the synthesis of Fcγ receptor may be accelerated by the signal resulting from the binding of modified α_2-macroglobulin to the mannose receptor. Thus, the binding parameters of Fcγ receptors were examined using HRP-conjugated mouse IgG (Fig. 5). The number of Fcγ receptors of activated macrophages was 4.6 times higher than the control value, and the presence of D-mannose was reduced to control levels. On the other hand, the binding constant was almost equivalent in each case. Consequently, the accelerated ingestion observed with modified α_2-macroglobulin resulted from an increase in the number of Fcγ receptors and not enhancement of the function of Fcγ receptors.

Yamamoto et al. [15, 19] have reported that inflammation products such as lysophosphatidylcholine and alkylglycerol activate mouse peritoneal macrophages and induce the phagocytosis of IgG-opsonized SRBCs via Fcγ receptors on macrophages. We have reported that lectin-like receptors having specificity for *N*-acetyl-β-D-galactosamine are involved in activating the process of Fcγ receptor-mediated phagocytosis, and this activation came from overexpression of Fcγ receptors following the interaction of ligand, modified vitamin D-binding protein (DBP), and *N*-acetyl-β-D-galactosamine receptor on macrophage surface [16].

Taking these findings into consideration, lectins having specificity for mannose or *N*-acetyl-β-D-galactosamine on the macrophage surface are involved in the activation of Fcγ receptor-mediated phagocytosis of opsonized SRBCs induced by liposomes or lysophosphatidylcholine. Binding of modified α_2-macroglobulin and modified DBP to these lectins induces the synthesis of Fcγ receptors on the surface of macrophages, and lectins may be involved in signal transduction in macrophage activation.

ACKNOWLEDGMENTS

This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, and Culture of Japan.

REFERENCES

1. Auger, M.J., Ross, J.A. (1992) Macrophage surface receptors. In *Macrophage* (C.E. Lewis and J.O'D. Macgee, eds) IRL Press, New York, 16-27.
2. Kolb, H., Kolu-Bachofen, V. (1978) A lectin-like receptor on mammalian macrophages. *Biochem. Biophys. Res. Commun.* **85**, 678-683.
3. Roos, P.H., Hartman, H.J., Schlopper-Schafer, J., Kolb, H., Kolb-Bachofen, V. (1985) Galactose-specific receptors on liver cells. II. Characterization of the purified receptor from macrophages reveals no structural relationships to the hepatocyte receptor. *Biochim. Biophys. Acta* **847**, 115-121.
4. Kuster, J.M., Schauer, R. (1981) Phagocytosis of sialidase-treated rat erythrocytes: evidence for a two-step mechanism. *Hoppe-Seyler's Z. Physiol. Chem.* **362**, 1507-1514.
5. Jancik, J., Schauer, R. (1974) Sialic acid. A determination of the lifetime of rabbit erythrocytes. *Hoppe-Seyler's Z. Physiol. Chem.* **355**, 395-400.
6. Stahl, P.D. (1992) The mannose receptor and other macrophage lectins. *Curr. Opin. Immunol.* **4**, 49-52.
7. Morell, A.G., Gregoriadis, G., Scheinberg, I.H. (1971) The role of sialic acid in determining the survival of glycoprotein in the circulation. *J. Biol. Chem.* **246**, 1461-1467.
8. Lenten, L.V., Ashwell, G. (1972) The binding of desialylated glycoproteins by plasma membranes of rat liver. Development of a quantitative inhibition assay. *J. Biol. Chem.* **247**, 4633-4640.
9. Aramaki, Y., Murai, M., Tsuchiya, S. (1994) Activation of Fc receptor-mediated phagocytosis by mouse peritoneal macrophages following the intraperitoneal administration of liposomes. *Pharm. Res.* **11**, 518-521.
10. Aramaki, Y., Murai, M., Tsuchiya, S. (1993) The contribution of a serum component(s) modified by B cells to the mechanism for macrophage activation by liposomes. *Immunology* **80**, 253-258.
11. Hashimoto, K., Suzuki, I., Yadomae, T. (1991) Oral administration of SSG, a β-glucan obtained from *Sclerotinia sclerotiorum*, affects the function of Peyer's patch cells. *Int. J. Immunopharmacol.* **13**, 437-442.
12. Suzuki, I., Kitamura, K., Kiyono, H., Kurita, T., Green, R.R., McGhee, J.R. (1986) Isotype-specific immunoregulation: evidence for a distinct subset of T contrasuppressor cells for IgA responses in murine Peyer's patches. *J. Exp. Med.* **164**, 501-516.
13. Bianco, C., Griffin, F.M., Silverstein, S.C. (1975) Studies of macrophage complement receptors: alteration of receptor function upon macrophage activation. *J. Exp. Med.* **141**, 1278-1290.
14. Yagawa, K., Onoue, K., Aida, Y. (1979) Structural studies of Fc receptors. I. Binding properties, solubilization, and partial characterization of Fc receptors of macrophages. *J. Immunol.* **122**, 366-373.
15. Yamamoto, N., Homma, S. (1991) Vitamin D_3 binding protein (group-specific component) is a precursor for the macrophage activating signal factor from lysophosphatidylcholine-treated lymphocytes. *Proc. Natl. Acad. Soc. USA* **88**, 8539-8543.
16. Aramaki, Y., Murai, M., Tsuchiya, S. (1993) Contribution of *N*-acetyl-β-D-galactosamine-specific lectin to Fc receptor-mediated phagocytosis by mouse peritoneal macrophages. *Immunology* **79**, 403-407.
17. Barrett, A.J., Starkey, P.M. (1973) The interaction of α_2-macroglobulin with proteinases: characteristics and specificity of the reaction, and a hypothesis concerning its molecular mechanism. *Biochem. J.* **133**, 709-724.
18. Salvesen, G.S., Barrett, A.J. (1980) Covalent binding of proteinases in their reaction with α_2-macroglobulin. *Biochem. J.* **187**, 695-702.
19. Yamamoto, N., Kumashiro, R. (1993) Conversion of vitamin D_3 binding protein (group specific component) to a macrophage activating factor by the stepwise action of β-galactosidase of B cells and sialidase of T cells. *J. Immunol.* **151**, 2794-2802.