

# Inhibition of Phagocytosis of Complement C3- or Immunoglobulin G-coated Particles and of C3bi Binding by Monoclonal Antibodies to a Monocyte-Granulocyte Membrane Glycoprotein (Mo1)

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**ABSTRACT** Events that lead to phagocytosis of complement (C3)- or IgG-coated particles after their interaction with specific cell surface receptors are poorly understood. Two mouse monoclonal antibodies (an IgM and an IgG2a) to a human granulocyte-monocyte surface membrane differentiation antigen (Mo1) inhibited ingestion by granulocytes both of oil Red O particles opsonized with normal human serum or with IgG and of sheep erythrocytes sensitized with IgG. In addition, they specifically inhibited rosetting between phagocytes and sheep erythrocytes coated with C3bi, a fragment of the complement component C3, generated by cleaving C3b with C3b inactivator and  $\beta$ 1H protein. These monoclonal anti-Mo1 antibodies did not inhibit IgG Fc, C3b, or C3d receptor-mediated binding of erythrocytes coated with the respective proteins. The Fab fragment of the IgG2a monoclonal antibody inhibited noncytotoxic enzyme release from granulocytes when these cells were stimulated with zymosan coated with C3bi. Electrophoretic transfer of polymorphonuclear leukocyte detergent lysates to nitrocellulose, followed by immunofixation with monoclonal antibody, showed that these antibodies were directed to a 155,000-mol wt glycoprotein. This sur-

face membrane structure appears to be involved in Fc and C3 receptor-dependent phagocytosis and closely associated with the C3bi receptor.

## INTRODUCTION

Surface membrane receptors for two fragments (C3b and C3bi; 1-4) of the third component of complement (C3) and for the Fc region of IgG (5, 6) are present on phagocytic cells. Interactions between these receptors and their respective ligands lead to a number of important biologic functions such as immune adherence, phagocytosis, antibody-dependent cellular cytotoxicity, and histaminase and lysosomal enzyme release (1, 7-10). The mechanisms involved in coupling receptor ligand interactions with these various functions are poorly understood. The development of the monoclonal antibody technique by Kohler and Milstein (11) offers means of analyzing such complex interactions rigorously. Monoclonal antibodies to the C3b and Fc receptors have proved useful in elucidating the structure and function of these molecules (12, 13).

Monoclonal antibodies to other cell surface antigens were instrumental in defining specific surface markers and have been extensively used for cell phenotyping and in studies of cell differentiation. Most of these antibodies, however, are directed against antigens for which function has not been defined. Among these monoclonals are antibodies against a monocyte-granulocyte surface antigen named Mo1, which consists of two distinct noncovalently linked glycoproteins with

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molecular weights of 155,000 and 94,000, as evidenced by sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis (PAGE)<sup>1</sup> of antibody immunoprecipitates from monocytes under both reducing or nonreducing conditions (14).

We now report that two monoclonal antibodies (an IgM and an IgG2a) to Mo1 specifically inhibited binding of C3bi-coated sheep erythrocytes (Es) to human neutrophils or monocytes. The Fab fragment of the IgG2a antibody inhibited ingestion by polymorphonuclear leukocyte (PMN) of particles opsonized with C3. Ingestion of particles opsonized with IgG alone was also inhibited. Noncytotoxic release of granulocyte enzymes was inhibited by this Fab fragment when induced by C3bi-coated zymosan but not by phorbol myristate acetate (PMA). This antibody is directed against the 155,000-dalton fragment of the Mo1 antigen complex, as determined by the immunoblotting technique (15).

## METHODS

**Phagocytes, lymphocytes, and tumor cell lines.** Human neutrophils were isolated from venous blood by density gradient centrifugation on Ficoll-Hypaque, followed by hypotonic lysis of the pellet, as previously described (16). Monocytes were purified by incubating the mononuclear cell fraction (obtained from Ficoll-Hypaque centrifugation) on plastic petri dishes (17). B lymphocytes were purified from mononuclear cell fractions as previously described (17). 55% of the lymphocyte population had the B1 antigen (a surface antigen specific for B lymphocytes (18)). Human erythrocytes from random donors were washed six times in phosphate-buffered saline (PBS) containing 10 mM EDTA.

Human B lymphoblastoid cell line (Raji) and CEM T lymphoblastoid cell line were provided by Dr. H. Lazarus, Dana Farber Cancer Institute, Boston, MA.

Es in Alsever's solution were obtained from M. A. Bio-products (Walkersville, MD).

**Immunoglobulins and complement proteins.** IgG rabbit antibody to Es was bought from Cordis Laboratories, Miami, FL. IgM rabbit antiserum to Es was obtained by immunizing rabbits with Es stroma as described (19). Rabbit IgG antibody to mouse Ig was a gift of Dr. J. Griffin, Dana Farber Cancer Institute. Rat antimouse MAC1 monoclonal antibody was kindly provided by Dr. T. Springer, Dana Farber Cancer Institute.

Mouse monoclonal antibodies to Mo1 were obtained by immunizing BALB/c mice with human monocytes (17). The Fab fragments of monoclonal antibodies were prepared as described (20) and purity of fragments shown by SDS PAGE analysis. Human C3, C3b dimers,  $\beta$ IH, and C3b inactivator were purified as described (2, 21-22). C3 was further purified by sequential affinity chromatography on Sepharose to which the Ig fraction of rabbit antibodies to human C5,

$\beta$ IH, and IgG were previously linked with cyanogen bromide (2).

**Iodinations.** Monoclonal Fab fragments or rabbit IgG antimouse immunoglobulins were radiolabeled with <sup>125</sup>I (New England Nuclear, Boston, MA), using chloramine T (10-15:1 molar ratio) to a specific activity of 100,000 and  $4 \times 10^6$  cpm/ $\mu$ g, respectively (23). Fluid phase C3b dimers were iodinated with insolubilized lactoperoxidase and glucose oxidase (enzymobeads, Bio-Rad Chemicals, Richmond, VA) as described (2).

**Preparation of immunoglobulin and complement-coated particles.** Es were coated with a rabbit IgG antibody to Es by incubating Es with the antibody in various dilutions (1:300 to 1:1,200) in PBS for 15 minutes at 37°C, followed by several washes in the same buffer.

Es coated with C3b (EAC43b) were made as follows: Es were sensitized with rabbit IgM anti-Forssman antibody, guinea pig C1, human C4, guinea pig C2, and limited amounts of human C3 as described by Rapp and Borsos (19). C1 and C2 were then decay dissociated by incubation in veronal-buffered saline containing 10 mM EDTA for 2 h at 37°C. In some experiments, Es coated only with purified C3b (EsC3b) were made by trypsin treatment of purified C3 (0.6% wt/wt, 6 min, room temperature [RT]) in presence of Es, followed by addition of soybean trypsin inhibitor in four-fold molar excess to stop the reaction. EAC43b or EsC3b cells had ~8,000-10,000 C3b molecules per cell when quantitated using <sup>125</sup>I-C3. EAC43bi or EsC3bi were generated by treating the respective cells with purified C3b inactivator (5  $\mu$ g/ml) and  $\beta$ IH (50  $\mu$ g/ml) in PBS containing 1 mM diisopropyl fluorophosphate (Sigma Chemical Co., St. Louis, MD) for 1 h at 37°C. This treatment abolished the capacity of these cells to bind to the C3b receptor on human erythrocytes (24). Their content of C3c antigen, however, was not significantly decreased, as determined by a radiometric binding assay (25) using a monoclonal antibody to human C3c antigen (provided by Dr. J. D. Capra). More than 90% of C3c antigen was released from these cells when treated with 0.1  $\mu$ g/ml TPCK trypsin (Millipore Corp., Freehold, NJ) in PBS for 45 min at 37°C, indicating that the predominant C3 fragment present is C3bi (26). EAC43d were obtained by treating EAC43bi with 0.1  $\mu$ g/ml trypsin in PBS for 45 min at 37°C. These cells did not form rosettes with human PMN, but strongly rosetted with Raji cells.

Zymosan A (Sigma Chemical Co.) was boiled in normal saline for 10 min. C3b-coated zymosan (ZC3b) was made as follows: Zymosan (15 mg) was pelleted and C3 (1 mg) and TPCK trypsin were added (0.6% wt/wt) in a total vol of 0.5 ml PBS for 6 min at RT. Further C3b was deposited by using the nickel-stabilized C3 convertase, as described (27). C3bi-coated zymosan (ZC3bi) was made by incubating ZC3b in 1 ml of normal human plasma (extensively preabsorbed with washed zymosan) for 16 h at 37°C. This resulted in >90% release of C3c antigen after trypsin treatment of washed particles, indicating the presence of the C3bi fragment. This prolonged incubation was necessary, because of the relative insensitivity of C3b deposited on zymosan to C3b inactivator cleavage (28).

Opsonized oil red O (ORO) and ORO coated with tetanus toxoid (Massachusetts Public Health Biologic Lab.) and human antitetanus toxoid immune globulin (Lederle Laboratories, Pearl River, NY) were prepared exactly as described (7, 29). Opsonized (C3 coated) ORO particles were used immediately or frozen at -90°C and used within 3 wk.

**Rosette formation.** 25  $\mu$ l of human leukocytes (PMN, monocytes, Raji cells, or B lymphocytes) at  $4 \times 10^6$ /ml in

<sup>1</sup> Abbreviations used in this paper: Es, sheep erythrocyte; HSA, human serum albumin; ORO, oil red O; PAGE, polyacrylamide gel electrophoresis; RT, room temperature.

0.025 M veronal buffer, containing 3.2% dextrose, 1% human serum albumin, 0.2% sodium azide, and 1 mg/ml soybean trypsin inhibitor (Millipore Corp.) were incubated with serial dilutions of immune or nonimmune ascites (or purified monoclonal anti-Mo1 and control antibodies) for 20 min at RT. 25  $\mu$ l of indicator cells ( $2 \times 10^6$ /ml) were added and the mixture incubated at 37°C for 30 min. To assess rosette formation, the cell mixture was gently resuspended and a drop placed on a hemocytometer; 400 human cells were then examined. Binding of three or more Es to a leukocyte was considered a rosette.  $^{125}$ I-C3b dimer binding to human erythrocytes was carried out as described (2).

**Assays of phagocytosis.** Ingestion of opsonized or IgG-coated ORO particles was performed as follows: Phagocytes ( $8 \times 10^6$  cells in 200  $\mu$ l) were preincubated in PBS alone or various concentrations of monoclonal anti-Mo1 or control antibodies. After 30 min at RT, volume was brought up to 800  $\mu$ l with Krebs-Ringer phosphate (KRP) buffer (pH 7.4). 400  $\mu$ l of cell suspension was incubated with 200  $\mu$ l of serum opsonized ORO or 125  $\mu$ l of IgG-coated ORO in the presence of 500  $\mu$ l of KRP or PBS containing 1 mM N-ethyl maleimide. Cells and opsonized particles were prewarmed for 5 min at 37°C before mixing. Afterward, the reaction mixture was incubated at 37°C for 5 min with continuous agitation. The reaction was stopped by adding 1 ml of PBS containing 1 mM N-ethyl maleimide to each tube, followed by two washes. The rate of ingestion was quantitated as described (7).

Ingestion of Es IgG by PMN preincubated with the Fab fragment of anti-Mo1 (5  $\mu$ g/ $10^6$  cells) or control antibodies was performed in suspension as described (29). Quantitation of phagocytosis was done by counting 200 Wright stained PMN under light microscopy. The percentage of cells ingesting one or more erythrocytes was determined. Alternatively, erythrophagocytosis was quantitated using  $^{51}$ Chromium-labeled Es (17) sensitized with IgG.

**Degranulation.** Enzyme release from PMN in response to zymosan coated with C3bi was measured as described by Zeiger et al. (30). Briefly, 100  $\mu$ l PMN at a concentration of  $5 \times 10^6$ /ml in Tris buffer containing 0.1% human serum albumin (HSA), 0.0006 M calcium, and 0.001 M magnesium were incubated at RT with buffer alone or the Fab fragment of monoclonal antibodies to Mo1, PLT1 (31), J5 (32), or Mo5 (33) antigens for 25 min. ZC3bi (6 particles/cell), or PMA (1  $\mu$ g/ml) were added to the cells in a total volume of 150  $\mu$ l and incubated for 30 min at 37°C in a shaking water bath. PMN were then pelleted by centrifugation at 9,000 g for 1 min at 4°C. The supernatant fluid samples as well as supernatant from Triton X-100 solubilized untreated PMN, buffer-treated PMN, and PMN treated with the separate monoclonal antibodies alone were assayed for lysozyme (34),  $\beta$ -glucuronidase (35), lactic dehydrogenase (36), and histaminase (9). The percentage of total enzyme activity released into the medium during activation was calculated. The percentage of specific release was calculated by subtracting spontaneous release in buffer alone from the release induced by C3-coated zymosan or PMA.

**Quantitation of Mo1 antigen on cells.** Leukocytes (PMN, monocytes, T cells, B cells) at  $3 \times 10^7$ /ml or human erythrocytes ( $3 \times 10^6$ /ml) in PBS containing 1% HSA were used. 75  $\mu$ l of each cell type were incubated with increasing concentrations of  $^{125}$ I-Fab anti-Mo1 in the absence or presence of a 70-fold molar excess of the unlabeled ligand. After 1 h on ice, 45- $\mu$ l aliquots in replicate were placed on a mixture of diethyl and dibutyl phthalate (6:4 ratio) (2) and centrifuged for 1 min at 4°C in an Eppendorf microfuge (Brinkmann Instruments, Inc., Westbury, NY) to separate free from

bound radiolabeled ligand. Specific binding, the amount of ligand bound (in nanograms), and bound/free ratios were then determined. The number of Mo1 sites per cell was estimated with Scatchard plots (37).

**Isolation of Mo1 from  $^{125}$ I-labeled PMN with affinity chromatography.** PMN were treated with diisopropyl fluorophosphate, surface labeled with iodogen (Pierce Chemical Co., Rockford, IL), and solubilized with Nonidet P-40 (NP-40) as previously described (29). F(ab')<sub>2</sub> anti-Mo1 antibody was covalently linked to Sepharose CL 6B using cyanogen bromide. 250  $\mu$ l of PMN lysate (equivalent to  $25 \times 10^7$  cells) were preabsorbed with Sepharose (100  $\mu$ l of resin) for 2 h at 4°C. The resin was pelleted by centrifugation and the supernatant incubated with 1 ml of anti-Mo1 Sepharose for 14 h at 4°C. The resin was then poured into a column, washed with 10 vol of PBS containing 2 mM phenyl methyl sulfonyl fluoride and 0.1% NP-40, and eluted with 0.5 N acetic acid containing 0.1% NP-40 (in 1-ml fractions) and the pH was immediately neutralized with 2 M Tris base to pH 7.5. One peak of radioactivity was eluted and analyzed by SDS PAGE (38). No radioactive peak was obtained with a control Sepharose column to which nonimmune F(ab')<sub>2</sub> mouse Ig was covalently linked.

**Electrophoretic blotting of PMN membrane proteins followed by immune fixation with monoclonal antibodies.** 100- $\mu$ l aliquots of PMN lysates were electrophoresed on SDS PAGE, followed by electrophoretic transfer of the proteins to nitrocellulose paper, as described (15). Immune ascites containing monoclonal antibody to Mo1 or nonimmune ascites were then incubated with the nitrocellulose strips at appropriate dilutions for 48 h at 37°C. After washing these with buffer,  $^{125}$ I-labeled rabbit IgG to mouse Ig was added and incubated for 1 h at 37°C. Strips were then extensively washed, dried, and exposed to Kodak Industrex C film and developed using a Kodak automated developer (Eastman Kodak Co., Rochester, NY).

## RESULTS

**Inhibition of C3bi binding to phagocytes by anti-Mo1 monoclonal antibodies.** Anti-Mo1 monoclonal antibodies inhibited rosette formation between PMN or monocytes and EAC43bi cells in a dose-dependent manner (Fig. 1). Purified IgM anti-Mo1 produced 50% inhibition of rosetting between PMN or monocytes and EAC43bi at 3.2  $\mu$ g/ml and 4.5  $\mu$ g/ml, respectively. Purified IgG2a anti-Mo1 produced 50% inhibition at 5.7  $\mu$ g/ml for PMN and 8  $\mu$ g/ml for monocytes (data not shown). No inhibition was seen with a control mouse monoclonal IgM directed against human platelets (Fig. 1) or with mouse monoclonal antibodies directed against Mo5 or J5 (data not shown). IgM anti-Mo1 did not inhibit binding of fluid phase  $^{125}$ I-C3b dimer to C3b receptors on human erythrocytes (Fig. 1, inset). Further evidence for the specificity of inhibition of C3bi binding to cells by IgM anti-Mo1 is provided in Table I. The single concentration of the monoclonal antibody that totally inhibited C3bi receptor-dependent rosette formation by monocytes and granulocytes had no effect on rosette formation that

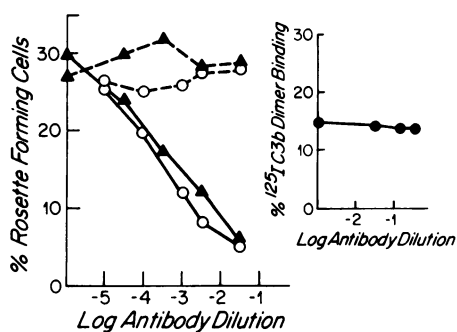


FIGURE 1 Dose-response relationship between the concentration of mouse monoclonal IgM anti-Mo1 (solid lines; log base 10) and the percentage of human monocytes ( $\blacktriangle$ ) or PMN ( $\circ$ ) forming rosettes with Es coated with C3bi. Interrupted lines represent the effect of the control monoclonal mouse IgM on rosette formation. Neither EAC4b (carrying >200 effective molecules C4b per cell) nor EAC4bi formed rosettes with human monocytes or granulocytes under these conditions. Inset: Effect of increasing concentrations of IgM anti-Mo1 on binding of  $^{125}\text{I}$ -C3b dimer to human erythrocytes. At a 100-fold molar excess of unlabeled ligand, labeled C3b dimer binding was 4% (nonspecific binding).

is dependent on interaction between C3b-, C3d-, or IgG-coated erythrocytes and C3b, C3d, or Fc receptors respectively. Equally notable is the finding that at doses of anti-Mo1 that almost completely block C3bi binding to PMN or monocytes, little inhibition of rosette formation between C3bi-coated Es and B lymphocytes or Raji cells was seen (Table I). Few if any rosettes were formed between human erythrocytes and C3bi-bearing Es (data not shown).

**Quantitation of Mo1 antigen on human cells.** To determine whether the differential inhibition of C3bi

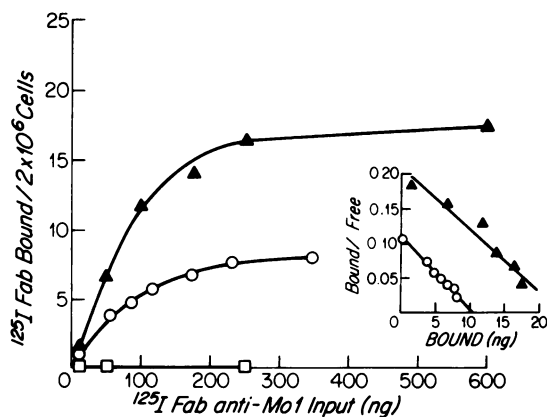


FIGURE 2 Quantitative analysis of binding of  $^{125}\text{I}$ -Fab anti-Mo1 (IgG2a) to human PMN ( $\circ$ ), monocytes ( $\blacktriangle$ ), and lymphoblastoid T cell line (CEM;  $\square$ ). Points represent the specific binding of duplicate determinations. Both monocytes and granulocytes bound  $^{125}\text{I}$ -Fab anti-Mo1 with equal affinity with an equilibrium dissociation constant ( $K_d$ ) of 14 and 10 nM, respectively. Nonspecific binding was <5% of total binding.

binding to human cells reflected differing numbers of antigenic sites, we quantitated the uptake of the  $^{125}\text{I}$ -labeled Fab fragment of the IgG2a anti-Mo1 antibody on various human cells (Fig. 2). There were 65,000 sites/PMN, 140,000 sites/monocyte, ~9,000 sites/cell in the B lymphocyte-enriched preparation, <100 sites/human erythrocyte, and <2,000 molecules/Raji cell. No specific binding was noted on a lymphoblastoid T cell line (Fig. 2). No significant inhibition of  $^{125}\text{I}$ -Fab anti-Mo1 binding to human PMN was seen when these cells were preincubated with 400-fold molar excess of unlabeled anti-MAC1 antibody (data not shown).

TABLE I  
Effect of IgM Anti-Mo1 on Rosette Formation between Human Cells and C3b-, C3bi-, C3d-, or IgG-coated Es

	EAC43b		EAC43bi		EAC43d		ElgG	
	-	+	-	+	-	+	-	+
PMN	50	51	28	3	0	0	70	73
Monocytes	65	62	40	3	0	0	79	80
B lymphocytes*	25	24	28	20	5	4	NT†	
Cell lines								
Raji cells	32	37	32	25	35	37	NT	
Lymphoblastoid T cells (CEM)	0	NT	0	NT	0	NT	NT	

Dose of IgM anti-Mo1 used was 1/10 dilution of immune ascites. -/+ refer to presence of monoclonal IgM control or anti-Mo1, respectively. All values represent mean of duplicate determinations.

\* 55% B1 positive cells.

† NT, not tested.

**Effect of anti-Mo1 on zymosan C3bi-dependent enzyme release from PMN.** To further assess the effect of anti-Mo1 on other C3-dependent functions, PMN were preincubated with Fab anti-Mo1 or with control Fab fragments to two other surface antigens (Mo5 and J5), then exposed to zymosan coated with C3bi (ZC3bi) and enzyme release was detected as described in Methods. ZC3bi-induced lysozyme,  $\beta$ -glucuronidase, or histaminase release were inhibited by anti-Mo1 in a dose-dependent manner (Figs. 3 and 4). Specific release of histaminase,  $\beta$ -glucuronidase, and lysozyme in the absence of the anti-Mo1 antibody were 45, 8, and 9%, respectively. No inhibition was seen with control monoclonal antibodies (Fig. 3). Anti-Mo1 did not significantly inhibit PMA-induced histaminase, or  $\beta$ -glucuronidase enzyme release (data not shown).

**Effects on phagocytosis.** Fab anti-Mo1 inhibited serum-opsonized (C3-coated) ORO ingestion by PMN (Fig. 5 A). Half-maximal inhibition was seen at 8.5  $\mu$ g/ml. No inhibition was seen with control Fab anti-Mo5 antibody. Similarly, the Fab fragment of monoclonal anti-Mo1 inhibited ingestion of IgG-coated ORO in a dose-dependent manner (Fig. 5 B). One-half-maximal inhibition was seen at an antibody concentration of 6  $\mu$ g/ml. Mouse ascites containing monoclonal anti-Mo1 or Fab fragment of purified anti-Mo1 IgG2a specifically inhibited phagocytosis by PMN of Es sensitized with IgG (at subagglutinating doses); (Table II). Maximal inhibition was seen at 1:100 dilution of immune ascites or 30  $\mu$ g Fab anti-Mo1/ml and ranged between 33 and 52%, depending on the

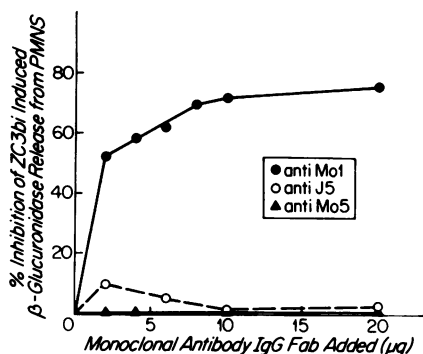


FIGURE 3 Effect of Fab anti-Mo1 (IgG2a) on ZC3bi-induced  $\beta$ -glucuronidase release from PMN. 50% inhibition was produced at 10.6  $\mu$ g/ml. No inhibition was seen by Fab anti-Mo5 or Fab anti-J5 monoclonal antibodies. Similar results were obtained in three other experiments. Specific release induced by ZC3bi alone was 8% of total (mean of duplicate determinations). Total enzyme activity was 54.7 nmol of phenolphthalein converted/h per  $10^7$  cells. No specific enzyme release was induced by any of the Fab fragments alone (data not shown).

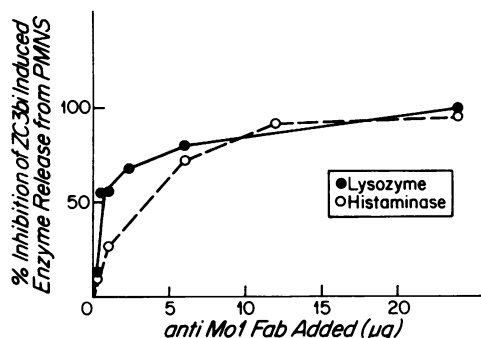


FIGURE 4 Inhibition of ZC3bi-dependent histaminase release (O) and of lysozyme release (●) from PMN by Fab anti-Mo1 (IgG2a). 50% inhibition of histaminase or of lysozyme release occurred at 20 and 5  $\mu$ g/ml of Fab anti-Mo1, respectively. Specific histaminase release in response to ZC3bi was 45% of total. Total histaminase activity was 55 pmol/h per  $10^7$  PMN. No specific inhibition was seen by Fab anti-Mo5 at similar concentrations. ZC3bi-induced specific lysozyme release was 9% of total. Total enzyme activity (expressed as  $\Delta$ OD540/min per  $10^7$  cells) was 0.344.

method used to quantitate ingestion (Table II). Higher degrees of inhibition (up to 100%) were obtained with erythrocytes coated with smaller number of IgG molecules (data not shown).

**Anti-Mo1 monoclonal antibody is directed to an epitope present on the 155,000-dalton fragment of the Mo1 antigen complex.** Anti-Mo1 immunoprecipitated two noncovalently linked membrane glycoproteins from monocytes (14). Purification of Mo1 from  $^{125}$ I-labeled granulocytes revealed a similar structure (Fig. 6). To determine against which of the two glycoproteins the anti-Mo1 antibodies were directed, PMN lysates were first electrophoresed on SDS PAGE, followed by immunoblotting, as described in Methods. As shown in Fig. 6, only the 155,000-dalton glycoprotein was seen after immunofixation with anti-Mo1 IgG2a. When purified  $^{125}$ I-Mo1 was electrophoresed on SDS polyacrylamide gel and then transferred to nitrocellulose, both the 155,000- and 94,000-radiolabeled bands were seen on nitrocellulose paper, as determined by radioautography (data not shown). These data indicate that the epitope recognized by the monoclonal anti-Mo1 antibody is located on the larger glycoprotein of the Mo1 complex.

## DISCUSSION

Mo1 antigen is a surface membrane glycoprotein present on granulocytes, monocytes, and null cells and was defined by monoclonal antibodies (17). The data presented in this paper indicate that the 155,000-dalton subunit of Mo1 is closely associated with C3bi receptor

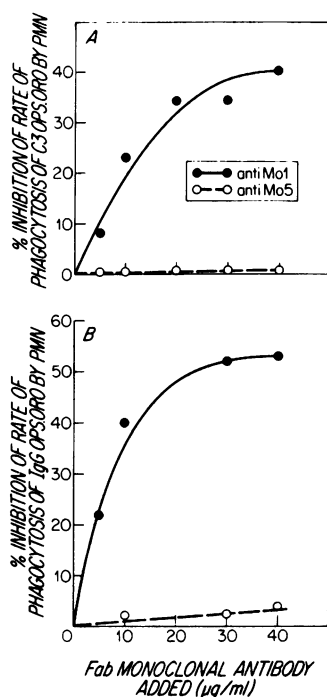


FIGURE 5 (A) Inhibition of C3-dependent phagocytosis by Fab anti-Mo1 (IgG2a). PMN were preincubated with increasing concentrations of anti-Mo1 Fab or control Fab (anti-Mo5) for 30 min at RT. Opsonized (C3 coated) ORO was then added and reaction carried out for 5 min at 37°C. Anti-Mo1 Fab produced half-maximal inhibition of C3-dependent phagocytosis at 8.5 µg/ml. The rate of ingestion in the absence of antibodies was 0.08 mg ORO/min per 10<sup>7</sup> PMN (mean of duplicate determinations). Similar data were obtained in four other experiments. (B) Dose-dependent inhibition by anti-Mo1 (IgG2a) of ingestion of IgG-coated ORO particles by PMN. Anti-Mo1 Fab produced half-maximal inhibition of phagocytosis at 6 µg/ml. The rate of ingestion in the absence of antibodies was 0.07 mg ORO/min per 10<sup>7</sup> PMN (mean of duplicate determinations). Similar results were seen in two other experiments.

functions and with a function dependent on C3bi and/or Fc receptors; i.e., phagocytosis. Evidence that suggests a relation between Mo1 and the C3bi receptor is based on the following findings: (a) anti-Mo1 specifically inhibited C3bi- (but not C3b-, C3d-, or IgG-) dependent rosette formation. (b) Enzyme release from PMN was inhibited by anti-Mo1 when release was induced by zymosan coated with C3bi, but not with PMA. (c) Fab anti-Mo1 inhibited ingestion of C3-opsonized ORO particles by PMN. These particles are coated with C3bi as well as C3b fragments (39).

Recently, Beller et al. (40) reported specific inhibition by a monoclonal antibody to a mouse macrophage surface antigen (MAC1) of rosette formation

TABLE II  
Effect of Anti-Mo1 on Phagocytosis of IgG-coated Es by PMN

	Sensitized erythrocyte*	
	Es IgG	<sup>51</sup> Cr-Es IgG
	% PMN ingesting	cpm/10 <sup>6</sup> PMN
Buffer alone	63, 69†	NT§
Ascites <sup>  </sup>		
Anti-Mo1	4, 4	358±44 (297-444)¶
Anti-Mo5	65, 69	1079±118 (928-1313)
Fab fragment		
Buffer alone	59, 68	
Anti-Mo1	35, 36	391±21.5 (362-433)
Anti-J5	69, 70	NT
Anti-Mo5	66, 68	1291±183 (930-1526)

\* Es incubated with 1:400 dilution of rabbit IgG anti-Es antibody for 15 min at 37°C.

† Values represent duplicate determinations.

§ Not tested.

<sup>||</sup> PMN (10<sup>6</sup> cells) were preincubated with 2 µl of ascites in a total volume of 200 µl for 20 min at RT. 50 µl of Es IgG (2 × 10<sup>8</sup>/ml) were added and incubation carried out for 60 min at 37°C with frequent mixing.

¶ Values represent mean±SE of triplicate determination. Figures in parenthesis represent the range. Approximately 15% of sensitized Es IgG were ingested by 10<sup>6</sup> PMN under these conditions.

between Es coated with C3bi and mouse or human PMN or macrophages. They concluded on the basis of these data that MAC1 most likely represents the C3bi receptor. Anti-MAC1 immunoprecipitated two non-covalently linked glycoproteins of mol wt 190,000 and 105,000 from mouse macrophages (41). No determination was made against which of these two peptides this antibody is directed and no structural data are available on the cross-reactive human antigen. The lack of significant inhibition by anti-MAC1 of <sup>125</sup>I-Fab anti-Mo1 binding to PMN indicate that these antibodies recognize different epitopes. The low affinity of anti-MAC1 to the human antigen (42) did not allow a more direct sequential immunoprecipitation analysis to determine whether both anti-MAC1 and anti-Mo1 are directed to the same surface antigen. Our data, although similar in regard to inhibition of C3bi-dependent rosetting and molecular weight of surface antigen, provide evidence that anti-Mo1 is not simply directed against the C3bi receptor, in as much as it interferes with phagocytosis dependent on IgG as well as C3bi.

C3bi receptors were reported to be present not only on phagocytes, but also on B lymphocytes or lym-

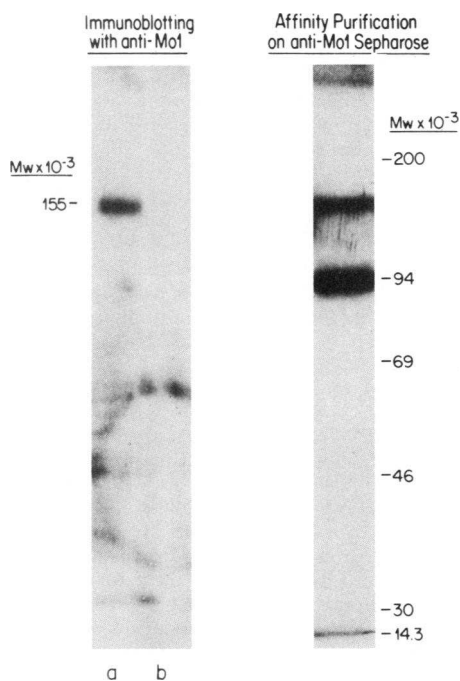


FIGURE 6 SDS PAGE on 7.5% gels of  $^{125}\text{I}$ -Mo1 isolated from  $^{125}\text{I}$ -labeled PMN as described in Methods. Two radiolabeled bands with mol wt 155,000 and 94,000 are seen (right lane). Left lane shows the immunofixation pattern seen when PMN lysate (containing membrane proteins) was electrophoresed on SDS PAGE, transferred to nitrocellulose, incubated with anti-Mo1 (a) or nonimmune ascites (b) and  $^{125}\text{I}$ -labeled rabbit anti-mouse Ig.

phoblastoid B cell lines (Raji) and human erythrocytes based on binding of Es or microspheres coated with C3bi to these cells (4). The very low specific binding of  $^{125}\text{I}$ -Fab anti-Mo1 to B lymphocytes or Raji cells, despite the normal rosetting with C3bi-coated erythrocytes, may suggest that Mo1 and C3bi receptor are different molecules. Alternatively, most of the rosetting that takes place between lymphocytes and C3bi-coated particles could be mediated by the C3d receptor (4). Preliminary data supporting the first possibility is that granulocytes defective in Fc- and C3-dependent phagocytosis (29) are deficient in Mo1 (by fluorescent-activated cell sorting analysis), but rosette normally with C3bi-coated sheep erythrocytes (43).

Ascites containing IgG monoclonal anti-Mo1 antibody inhibited specifically the ingestion by PMN of particles coated with IgG. This effect was also seen with Fab anti-Mo1 and at concentrations that were only four to six times greater than amounts needed to saturate Mo1 binding sites on PMN at 0°C. Moreover,

the purified IgG fraction of anti-Mo1 at 50  $\mu\text{g}/\text{ml}$  did not inhibit binding of IgG-coated Es to PMN. These findings indicate that the inhibition of IgG-dependent phagocytosis by anti-Mo1 is not likely due to steric interference with the Fc receptor. Maximal inhibition of ingestion of IgG-coated particles by PMN was 50%, even at high concentrations of anti-Mo1. One possible explanation for these data is that ligand (Fc) may displace anti-Mo1 at high IgG input, thereby overcoming in part the inhibitory effect. Alternatively, at high IgG input, an IgG-dependent but Mo1-independent mechanism for uptake may be revealed. At low IgG input, nearly 100% inhibition of Fc-dependent uptake is produced by the presence of anti-Mo1.

Thus, a monoclonal antibody directed to a surface glycoprotein (the 155,000-dalton subunit of the Mo1 antigen) on phagocytes specifically inhibited binding of C3bi-coated erythrocytes. It also inhibited ingestion of IgG- or C3-coated particles by PMN. This might be due to one or more of the following possibilities. (a) The 155,000-dalton subunit is the C3bi receptor. This would explain the specific interference by monoclonal anti-Mo1 of C3bi rosette formation. It can not explain, however, the inhibition of phagocytosis of IgG-coated particles, unless Mo1 is itself involved in IgG-dependent phagocytosis. (b) This 155,000-dalton subunit of Mo1 may represent the structure on PMN involved in phagocytosis of particles coated with C3 (C3b or C3bi) and/or IgG. Binding of such particles to their respective receptors may elicit an activating signal for ingestion that is mediated by the 155,000-dalton glycoprotein. Inhibition of C3bi binding may be explained by steric inhibition. (c) The 155,000-dalton subunit may function as a lectinlike molecule in promoting contact or adherence between cells and particles whether these were activators or nonactivators of the alternative complement pathway. Interference with this function may inhibit the rate of phagocytosis of C3- or IgG-coated particles. We can not conclusively distinguish among these possibilities at present. The exact functional relationship of the 94,000-dalton glycoprotein that coprecipitates with the 155,000-dalton subunit is also unclear, since both monoclonal antibodies to Mo1 used in this study recognize an epitope on the 155,000-dalton subunit.

These data establish the functional properties of the 155,000-dalton subunit of Mo1 antigen. Further studies, however, aimed at isolation of the C3bi receptor by methods dependent on interaction with its ligand and comparison of the molecular species obtained with Mo1 will be needed before the precise relationship between Mo1 and specific cell surface receptors can be established.

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