Recognition of bacteria by phagocytes is crucial to host defenses against infection. The elimination of bacteria generally occurs by specific receptor-mediated endocytosis and intracellular digestion. In the immune host, phagocytosis by Fc receptors and complement receptors is mediated by the presence of IgG and certain complement components on the bacterial surface. In the absence of these opsonins, other mechanisms, including carbohydrate-lectin interactions and adherence-promoting receptors, provide important alternative means for recognition and ligation (1–3).

Two pathways of opsonin-independent phagocytosis use carbohydrate-mediated recognition mechanisms. First, lectin-like molecules on the surface of phagocytes can recognize specific carbohydrates on target particles. These receptors for carbohydrate include the β-glucan receptor, which recognizes activators of the alternative complement pathway (4), and the mannosyl/fucosyl receptor, which ligates mannose on the surface of zymosan particles and on Leishmania donovani (5–9). A second mechanism of opsonin-independent recognition involves the adherence of lectin-like substances on the surface of bacteria to carbohydrates on the surface of phagocytes. For example, many Gram-negative bacteria, such as Escherichia coli, Klebsiella, and certain species of Salmonella and Shigella possess mannose-specific surface adhesions by which they bind to mannose residues on phagocytic cells. When this binding involves appropriate cell surface receptors, it may trigger internalization of the receptor–ligand complex in a manner similar to that of an immune opsonin–receptor complex with subsequent killing of the pathogen (10–13).

The cell surface molecules with mannose residues available for binding to the mannose-specific adhesions on microbes have not yet been fully characterized. We have shown that the binding of Con A–treated erythrocytes (E–Con A) to freshly explanted human monocytes is dependent on the specific interaction of Con A with surface mannose on the phagocyte, and that Fcγ receptors on human monocytes mediate the internalization of these E–Con A (14). These observations led to the hypothesis that an Fc receptor, containing carbohydrate moieties with...
exposed mannose, may be the receptor responsible for the ingestion of nonopsonized bacteria with surface mannose-binding adhesions. In this study, we demonstrate that phagocytosis of E-Con A and nonopsonized E. coli by human polymorphonuclear leukocytes (PMN)\(^1\) is mediated by the Fcy receptor that expresses the 3G8 epitope (15). Thus, a specific Fcy receptor on human PMN may initiate opsonin-independent phagocytosis of bacteria through lectin-carbohydrate interactions.

**Materials and Methods**

**Preparation of neutrophils.** Peripheral blood, obtained by venipuncture from healthy volunteers, was drawn into heparinized syringes. Neutrophils (PMN) were separated by density gradient centrifugation on Ficoll-Hypaque (\(d = 1.119\)) followed by hypotonic lysis of contaminating erythrocytes. The PMN were resuspended in RPMI with 20% heat-inactivated IgG-free FCS serum (Gibco Laboratories, Grand Island, NY) at 5 \(\times\) 10\(^6\) cells/ml. Microscopic examination of the cells demonstrated >95% PMN.

To block surface Fc receptors, PMN and aggregated IgG (Agg-IgG), prepared as described by McDougall et al. (16) and used at final concentrations from 75–600 \(\mu\)g/ml, were incubated at 37°C for 30 min with occasional agitation (17). The control PMN were incubated with medium alone. Blockade of the PMN Fc receptor–ligand binding site was also achieved by incubating PMN with the murine IgG1 mAb 3G8 (kindly provided by Drs. Sally Clarkson and Jay Unkeless of The Rockefeller University, New York [15]) at 10 \(\mu\)g/ml at 37°C for 10 min with occasional agitation. The control PMN for these experiments were incubated with an irrelevant IgG1 murine mAb (MOPC 21; Sigma Chemical Co., St. Louis, MO) at 10 \(\mu\)g/ml. In certain experiments, PMN were incubated with 3G8 Fab fragments (250 \(\mu\)g/ml) for 10 min. The 3G8 Fab was prepared by papain digestion of 3G8 mAb and purified by Staph protein A affinity chromatography at pH 8.3 (18, 19). The 3G8 Fab preparation was shown to be free of intact IgG by silver stain analysis of SDS-PAGE gels (20). After incubation, the PMN were washed with PBS and resuspended at 5 \(\times\) 10\(^6\) cells/ml.

**Preparation of Particles.** Antibody-sensitized erythrocytes (EA) were prepared for each experiment as previously described (21). Bovine erythrocytes were washed and incubated with rabbit IgG anti-bovine erythrocyte antibody (Cappel Laboratories, Cochranville, PA) for 1 h at 37°C. The cells are washed four times in PBS and resuspended at 10\(^8\) cells/ml in RPMI and 20% FCS.

E-Con A were prepared for each experiment by a modification of the method of Goldman and Cooper (22) as previously described (14). Rabbit erythrocytes at 10\(^8\) cells/ml in PBS were incubated with Con A, 0.1 \(\mu\)g/ml, for 30 min at 37°C. The cells were then washed and resuspended at 10\(^8\) cells/ml in RPMI and 20% FCS.

Serum-treated zymosan A (STZ) was prepared for each experiment by incubating zymosan (Sigma Chemical Co.) at 10 mg/ml in serum from normal volunteers for 30 min at 37°C with vortexing at 5-min intervals (23). The suspension of zymosan was washed in PBS and resuspended at 5 mg/ml in RPMI with 20% FCS.

**Assay of Phagocytosis of Particles.** To assess internalization of target particles, PMN in RPMI with 20% FCS (100 \(\mu\)l) were combined with EA, E-Con A or STZ (100 \(\mu\)l). For the assay of E-Con A phagocytosis, Con A was added to the incubation mixture to a final concentration of 5 \(\mu\)g/ml (14, 22). After centrifugation at 44 g for 5 min, the PMN-particle mixtures were incubated at 37°C for 30 min. In certain experiments, monosaccharides were added to the erythrocyte-PMN incubation mixture at a final concentration of 0.15 M for the duration of the incubation. The monosaccharides included a-methylmannoside, D-mannose, glucose, galactose, and N-acetylgalactosamine (Sigma Chemical Co.). After resuspension, the cells were washed twice with ammonium chloride erythrocyte

\(^1\) Abbreviations used in this paper: Agg-IgG, heat-aggregated human IgG; EA, IgG-sensitized bovine erythrocytes; E-Con A, concanavalin A–treated rabbit erythrocytes; Fc receptor, Fcy receptor; PI, phagocytic index; PMN, polymorphonuclear leukocyte; STZ, serum-treated zymosan.
lysis solution to remove noninternalized erythrocytes. The PMN incubated with STZ were washed only with PBS. After a final wash with PBS, the cells were resuspended in 50 μl of PBS. Visual light microscopic examination of the resuspended cell pellet was used to quantitate the percent of PMN with internalized target particles. At least 400 cells were counted for each condition. The cells were examined without knowledge of the PMN pretreatment condition. The data are expressed as percent of PMN ingesting one or more particles. In some experiments, the data are expressed as phagocytic index (the number of ingested particles per 100 PMN). <1% PMN ingest untreated bovine or rabbit cells.

Erythrocyte Binding Assay. PMN (5 × 10⁶/ml in PBS) in the presence of cytochalasin B (5 μg/ml) were incubated with or without Agg-IgG (300 μg/ml) or 3G8 Fab (250 μg/ml) for 5 min at 37°C. The cells were washed, resuspended in PBS with cytochalasin B, and combined with EA or E-Con A at a 100:1 ratio of erythrocytes to PMN. After centrifugation at 44 g for 3 min, the PMN-erythrocyte mixtures remained at room temperature for 30 min. The percentage of PMN with at least one adherent erythrocyte was determined by light microscopy after counting at least 400 cells.

Assay of Attachment and Phagocytosis of E. coli. E. coli strain 3092, a K12 derivative obtained from the American Type Culture Collection (Rockville, MD), was grown in trypticase soy broth (Scott Laboratories, Inc., Fiskeville, RI) and frozen in 20% glycerol in small aliquots, which were stored at −70°C. For each experiment, E. coli were inoculated into trypticase soy broth and cultured 24 h at 37°C to attain stationary phase of culture to allow for maximum piliation (12, 24). These growth conditions yield smooth E. coli that possess mannose-sensitive adhesions. Mannose binding capacity, shown by other investigators to be related to the presence of type I pili, was demonstrated by the capacity of this E. coli isolate to agglutinate guinea pig erythrocytes (24, 25) and saccharomyces cerevisiae yeast cells (10) as determined by the methods of Eden and Hansson (24) and Bar Shavit et al. (10), respectively. This agglutination was specifically inhibited by 0.20 M α-methylmannoside.

Before use in the assay, E. coli were washed in PBS twice and resuspended in HBSS with gelatin 0.1% (Difco Laboratories Inc., Detroit, MI). Equal volumes of E. coli at 10⁹/ml in HBSS with 0.1% gelatin and PMN at 5 × 10⁶/ml in HBSS with 0.1% gelatin were combined. In certain experiments, monosaccharides (see above) were added to the mixture to achieve a final concentration of 0.15 M. After agitation, 100 μl of PMN–bacteria mixture was placed on acid-washed circular glass coverslip (12-mm diameter; Fisher Scientific Co., Pittsburgh, PA) and incubated for 30 min at 37°C. Coverslips were then gently washed with HBSS, air dried, fixed with methanol, and stained with Giemsa. The association (attachment and/or phagocytosis) of E. coli with PMN was assessed by light microscopy. At least 400 cells were counted per slide. The data are expressed as percent association (the percent of PMN with attached and/or ingested E. coli) or association index (the number of attached and/or ingested E. coli per 100 PMN).

Data Analysis. The percentage phagocytosis or association of different particles by PMN after the various pretreatments was compared with untreated PMN by the paired t-test (two-tailed). For statistical analysis, a probability of 0.05 was used to reject the null hypothesis.

Results

Fc Receptors Mediate the Phagocytosis of E–Con A. In initial experiments, we demonstrated that Con A–treated erythrocytes bind to PMN and that this binding triggers internalization. The percent of neutrophils that ingested E–Con A and IgG-sensitized erythrocytes was similar (60 ± 19% vs. 73 ± 19%, respectively) (Fig. 1), although the phagocytic index was higher for EA (177 EA internalized per 100 PMN vs. 66 E–Con A per 100 PMN).

To examine the possibility that Fcγ receptors participate in E–Con A ingestion in PMN as has been demonstrated in monocytes (14), we quantitated E–Con A phagocytosis by PMN after Fc receptor blockade induced by aggregated human
IgG. Incubation of PMN with Agg-IgG (75–600 μg/ml) results in decreased availability of Fc receptors on the cell surface (17). As shown in Fig. 1, Fc-mediated phagocytosis of EA was inhibited by 97% in neutrophils preincubated with Agg-IgG. Pretreatment with Agg-IgG also caused a 90% inhibition of internalization of E-Con A (control vs. Agg-IgG: 60 ± 19% phagocytosis vs. 6 ± 4%, p < 0.001; Fig. 1). These decreases in the percentage of PMN-ingesting particles were comparable to the decreases in the phagocytic indices (control vs. Agg-IgG: 177 vs. 1.3 EA internalized per 100 PMN; 66 vs. 5.1 E-Con A internalized per 100 PMN). Fc receptor–independent endocytosis under these conditions was unaffected as demonstrated by the normal ingestion of serum-treated zymosan (Fig. 1), which is mediated by CR3 (the C3bi receptor) (26). Thus, the decrease in phagocytosis of both EA and E-Con A by Agg-IgG-treated PMN was a reflection of a specific decrease in an Fcγ receptor–mediated process rather than a generalized suppression of phagocytic activity.

To determine whether the participation of the Fc receptor in the internalization of E-Con A is as the principal Con A binding cell surface molecule or as the primary cell surface receptor mediating phagocytosis, we examined the effect of Fc receptor modulation on binding alone. Phagocytosis was abrogated by cytochalasin. When phagocytosis of E-Con A was blocked by treating PMN with Agg-IgG, binding of E-Con A to the PMN was clearly demonstrable. The percent of PMN binding E-Con A was not decreased in PMN with modulated Fc receptors as compared to control cells (control vs. Agg-IgG: 24 ± 4% PMN with adherent E-Con A vs. 24 ± 7%) (Fig. 2). Under similar conditions, EA

![Figure 1](https://jem.rupress.org/)
binding to PMN was markedly inhibited (control vs. Agg-IgG: 56 ± 15% PMN with adherent EA vs. 13 ± 3%, p < 0.02). Thus, Fcγ receptor modulation inhibited E–Con A ingestion by blocking phagocytosis rather than by affecting E–Con A binding. These observations indicate that E–Con A bind to many PMN surface glycoproteins but that the Fcγ receptor is the membrane protein that promotes ingestion.

**Specific Monosaccharides Inhibit the Ingestion of E–Con A.** To test the hypothesis that the mechanism of IgG-independent ligation of Fc receptors by E–Con A involves binding of available mannose moieties on Fc receptors by the multivalent Con A, we examined various monosaccharides for their ability to specifically inhibit the phagocytosis of our lectin probe. A panel of monosaccharides, α-methylmannoside, d-mannose, glucose, galactose, and N-acetylglucosamine, each at a final concentration 0.15 M, were added to the mixture of PMN and E–Con A for the duration of the incubation. In the presence of α-methylmannoside and d-mannose, E–Con A internalization was significantly and substantially inhibited. α-methylmannoside and d-mannose inhibited phagocytosis by 81% (12 ± 11% phagocytosis vs. 62 ± 14%, p < 0.001) and by 69% (19 ± 9% vs. 62 ± 14%, p < 0.001), respectively (Fig. 3). Simultaneous determination of the phagocytosis of EA showed no corresponding inhibition by the monosaccharides studied. The degree of inhibition of E–Con A ingestion induced by the other monosaccharides tested (Fig. 3) paralleled the known affinities of these sugars for Con A and their capacity to inhibit other Con A–polysaccharide interactions: α-methylmannoside > d-mannose > d-glucose > N-acetylglucosamine > galactose (27-28). The lack of complete inhibition of phagocytosis by α-methylmannoside may reflect the lower affinity of monovalent binding of the monosaccharides to Con A compared with the potential for higher affinity multivalent binding of Con A to cell surface glycoproteins with multiple mannose residues.

Since the phagocytosis of EA was not substantially inhibited by the monosaccharides studies, carbohydrate-lectin interactions involving the available mannose
residues on the Fc receptor do not play a critical role in the interaction of the PMN Fc receptor and its classical ligand, the Fc region of IgG. In contrast, the specific and marked inhibition of E-Con A ingestion by α-methylmannoside and D-mannose indicates that attachment to mannose moieties on the PMN receptor triggers the internalization of E-Con A.

Modulation of Fc Receptors Inhibits the Association of PMN with E. coli and the Phagocytosis of E. coli. Previous studies (10–13) have shown that mannose-specific adhesions on type 1 fimbriae of E. coli mediate attachment to phagocytes and subsequent ingestion of nonopsonized bacteria. The (mannose-containing) surface glycoproteins to which the E. coli bind and which mediate internalization have not been characterized. Since the internalization of E-Con A is mediated by Fc receptors, we examined the possibility that Fc receptors might play a role in the attachment and internalization of nonopsonized E. coli.

Using the W3092 strain of E. coli, which has a smooth morphology and mannose-binding adhesions, we confirmed the findings of other investigators that the binding and phagocytosis of the E. coli by PMN in a serum-free system was specifically inhibited by α-methylmannoside and D-mannose (10–12). As shown in Fig. 4, percent of PMN with associated (bound and/or phagocytosed) E. coli was decreased by 89% in the presence of α-methylmannoside (6 ± 4%
association vs. 55 ± 15%, \( p < 0.001 \) and by 85% in the presence of d-mannose (8 ± 4% vs. 55 ± 15%, \( p < 0.001 \)). Even more striking, the association index for the control PMN was 392 bacteria per 100 PMN compared with 9 bacteria per 100 PMN in the presence of \( \alpha \)-methylmannoside (Fig. 5B). Incubation of the \textit{E. coli} and PMN mixture with other monosaccharides had minimal effects on the association of PMN and \textit{E. coli} (Fig. 4).

To establish the role of Fc receptors in the binding and ingestion of these \textit{E. coli}, we examined the effects of modulation of PMN Fc receptors with Agg-IgG on the percent association and association index of the \textit{E. coli}. Fig. 5 clearly shows that the percent of PMN with associated \textit{E. coli} and the number of organisms associated per phagocyte was markedly decreased in the cells preincubated with Agg-IgG. The percent of PMN with associated \textit{E. coli} was inhibited by 46% (control vs. Agg-IgG: 67 ± 15% association vs. 36 ± 14, \( p < 0.0001 \)) and the association index was inhibited by 64% (363 ± 139 bacteria per 100 PMN vs. 129 ± 38, \( p < 0.002 \)) (Fig. 6, A and B). These observations suggested that Fc receptors on PMN mediated the association (binding and/or phagocytosis) of PMN with nonopsonized \textit{E. coli} possessing mannose-binding adhesions.

As with the E-Con A probe, modulation of Fc receptors on PMN might inhibit the association of \textit{E. coli} by blocking either the attachment or the phagocytosis of the \textit{E. coli}. To attempt to distinguish between internalized bacteria and adherent organisms we postulated that most extracellular \textit{E. coli} were bound to PMN through lectin–carbohydrate interactions and would dissociate from the PMN in the presence of \( \alpha \)-methylmannoside leaving mainly the intracellular organisms. After the 30-min incubation for formation of the PMN–\textit{E. coli} complexes, they were incubated with 0.15 M \( \alpha \)-methylmannoside for 25 min at 37°C. This second incubation indeed resulted in a decrease in PMN–\textit{E. coli} association index for both control and Agg-IgG-treated PMN (no \( \alpha \)-methylmannoside, control vs. Agg-IgG: 347 ± 144 \textit{E. coli} per 100 PMN vs. 120 ± 31, \( p < 0.007 \)); \( \alpha \)-methylmannoside, control vs. Agg-IgG: 150 ± 68 \textit{E. coli} per 100 PMN vs. 43 ± 34, \( p < 0.004 \)). At least 60% of the associated \textit{E. coli} were extracellular in both the control and Agg-IgG-treated PMN, but use of \( \alpha \)-methylmannoside
FIGURE 5: The effects of α-methylmannoside and Agg-IgG on the association of E. coli with PMN. (A) PMN incubated with E. coli in control medium and stained as described in Materials and Methods. Arrows indicate examples of PMN with numerous extracellular and/or intracellular E. coli. (B) PMN incubated with E. coli in the presence of 0.15 M α-methylmannoside. Binding and ingestion of E. coli are blocked. (C) PMN pretreated with Agg-IgG and incubated with E. coli. Only occasional associated E. coli are present. Both percent association and number of organisms associated per PMN are significantly decreased. X 1,000
OPSONIN-INDEPENDENT LIGATION OF Fcγ RECEPTORS

FIGURE 6. The effects of modulation of the Fc receptor on binding and phagocytosis of E. coli. PMN were incubated with control medium, Agg-IgG, mIgGl, or 3G8 mAb for 10 min. After washing, E. coli were added, the mixture was layered on glass coverslips, and was incubated for 30 min. After the coverslips were fixed and stained, the percent of PMN with associated (attached and/or internalized) E. coli (A) and the association index (B) was determined by light microscopy. Phagocytic index (C) was calculated by subtracting the mean association index of cytochalasin B–treated PMN (132 bound E. coli per 100 PMN) from the association indices represented in B. * Agg-IgG vs. control, p < 0.0001; 3G8 mAb vs. mIgGl, p < 0.0001; ** Agg-IgG vs. control, p < 0.002; *** 3G8 mAb vs. mIgGl, p < 0.001.
incubation was unable to completely distinguish between the effects of Fc receptor modulation on binding and phagocytosis. Accordingly, PMN were treated with cytochalasin B (5 μg/ml) before and during the incubation with E. coli to prevent internalization so that the attachment component alone could be assessed. The attachment index of E. coli to control PMN (104 E. coli per 100 PMN) was similar to that of Agg-IgG-treated PMN (171 E. coli per 100 PMN), indicating that modulation of Fc receptors does not block the attachment of E. coli to PMN. The slightly greater attachment to Agg-IgG-treated PMN may result from binding of E. coli to mannose on the Agg-IgG on the PMN membrane.

Since blockade of Fc receptors did not decrease the attachment of E. coli to PMN but did decrease the association index that represents both attached and internalized bacteria, the effect of Fc receptor modulation must be at the level of phagocytosis. With this rationale, the phagocytic index of E. coli was calculated by subtracting the mean attachment index of a series of cytochalasin B–treated PMN experiments (132 E. coli per 100 PMN) from the association indices of both control and Agg-IgG-treated PMN (Fig. 6C). Using this determination of quantitative phagocytosis, it is clear that Fc receptor blockade completely inhibited the ingestion of E. coli by PMN (control vs. Agg-IgG: 231 ± 138 E. coli per 100 PMN vs. 0 ± 38, p < 0.002). These data indicated that E. coli bind to many different glycoproteins on the cell surface, but that Fc receptors are unique in mediating the internalization of these E. coli possessing mannose-binding adhesions.

The 3G8 Epitope-bearing Fc Receptor Mediates the Internalization of E–Con A and E. coli. Two distinct Fc receptors for the IgG have been demonstrated on freshly explanted, unstimulated human neutrophils, a 50–73-kD molecule and a 40-kD molecule (15, 29). The mAb 3G8 recognizes the 50–73-kD Fc receptor and has been shown to inhibit the binding of IgG-sensitized erythrocytes to PMN (15). To delineate which neutrophil Fc receptor mediates E–Con A ingestion, we studied the effect of the 3G8 mAb on the phagocytosis of E–Con A by PMN. Although the 50–73-kD Fc receptor has a low avidity for IgG, we controlled for possible nonspecific blocking of PMN Fc receptors by the Fc region of 3G8 mAb with MOPC 21, an irrelevant, subclass-matched, murine IgG1 mAb. Pretreatment of PMN with murine IgG1 did not inhibit ingestion of either EA or E–Con A. In contrast, incubation of PMN with 3G8 mAb (10 μg/ml) blocked internalization of E–Con A and EA by 73% (p < 0.002) and 91% (p < 0.001), respectively (Fig. 1). Similarly, pretreatment of PMN with the monovalent Fab portion of 3G8 caused a 73% and a 97% decrease in the percentage of phagocytosis of E–Con A and EA, respectively (Table 1). Fc receptor–independent phagocytosis was unaffected by pretreatment with either intact 3G8 mAb or with 3G8 Fab as demonstrated by normal ingestion of serum-treated zymosan (control: 91% phagocytosis; 3G8 mAb: 94% phagocytosis; 3G8 Fab: 94% phagocytosis). These data argued strongly that the Fc receptor bearing the 3G8 epitope mediates E–Con A ingestion. We could not, however, categorically exclude minor participation of the 40-kD Fc receptor in the ingestion of E–Con A.

While phagocytosis of E–Con A is blocked by modulation of the 3G8-bearing Fc receptor with 3G8 Fab, there was no inhibition of E–Con A binding (control
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TABLE I

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<tr>
<th>Pretreatment</th>
<th>Phagocytosis of:</th>
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<tr>
<td></td>
<td>EA</td>
<td>E-Con A</td>
</tr>
<tr>
<td>Control</td>
<td>82.5 ± 4.7</td>
<td>63.0 ± 7.0</td>
</tr>
<tr>
<td>3G8 mAb</td>
<td>6.0 ± 4.8</td>
<td>12.23 ± 6.8</td>
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<tr>
<td>P&lt;0.001*</td>
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<tr>
<td>3G8 Fab</td>
<td>2.8 ± 2.1</td>
<td>17.0 ± 5.9</td>
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PMN were pretreated with 3G8 mAb (10 µg/ml), 3G8 Fab (250 µg/ml), or control medium, and then incubated with EA or E-Con A for 30 min. Noninternalized erythrocytes were lysed. Phagocytosis by 500 PMN was quantitated by microscopy. Results are expressed as percent phagocytosis and represent the mean ± SD of four different experiments.

* Paired t-test vs. control, two-tailed.

vs. 3G8 Fab: 23 ± 6% PMN with adherent E-Con A vs. 24 ± 5%). Under similar conditions, EA binding to PMN was markedly inhibited (control vs. 3G8 Fab: 55 ± 8% PMN with adherent EA vs. 8 ± 3%) (Fig. 2). These results, confirming those using Agg-IgG-treated PMN, demonstrate that E-Con A bind to many surface glycoproteins but the Fc receptor bearing the 3G8 epitope is the predominant membrane protein that promotes ingestion.

To determine whether the mechanism of ingestion of the nonopsonized E. coli, is similar to that for E-Con A, we studied the effects of 3G8 mAb on the binding and phagocytosis of these E. coli. When PMN were pretreated with 3G8 mAb, there was a 42% decrease in the percentage of PMN with associated E. coli and a 48% decrease in the association index compared with those treated irrelevant murine IgG1 mAb (Fig. 6, A and B). In contrast, the attachment of E. coli, as assessed in cytochalasin B–treated cell, was not affected by blockade of Fc receptors (3G8 mAb vs. IgG1: 119 E. coli per 100 PMN vs. 134 E. coli per 100 PMN). Calculation of the phagocytic index revealed a 92% decrease in E. coli ingestion in PMN treated with 3G8 mAb (Fig. 6C). This inhibition, comparable to that induced by Agg-IgG, demonstrated that the Fc receptor recognized by 3G8 mAb mediates the internalization of these nonopsonized E. coli. As with the data pertaining to E-Con A, we could not categorically exclude a minor role for a second receptor since Fc blockade did not totally block phagocytosis of E. coli.

Discussion

In the present study, we demonstrated that the ingestion of E-Con A and nonopsonized E. coli by PMN is mediated by Fc receptors recognized by the mAb 3G8 and is dependent upon lectin-carbohydrate interactions. Modulation of the Fc receptors by preincubating with Agg-IgG or with 3G8 anti–Fc receptor mAb markedly inhibited internalization of both E-Con A and E. coli (Figs. 1, 5, and 6). The experiments in which we used the 3G8 Fab to specifically ligate the 53–70 kD PMN Fc receptor demonstrated that this receptor is primarily respon-
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sible for the ingestion of E-Con A (Table I). Thus, it is apparent that ligands other than IgG can gain access to the classical immune receptor for IgG.

E-Con A and E. coli are recognized by the specific interaction of lectin (Con A) or lectin-like adhesions with glycoproteins on the cell membrane. Inhibition of association is induced only by α-methylmannoside and d-mannose (Figs. 3 and 4), the monosaccharides with the greatest affinity for Con A and other mannose-binding lectins (27, 28). As a model to explain Fc receptor-mediated ingestion of these nonopsonized particles, we propose binding of carbohydrate moieties on Fc receptors by the multivalent mannose-specific lectins (Con A) or adhesions (E. coli type I fimbriae) with subsequent crosslinking and triggering ingestion of the bound E-Con A or bacteria. Though E-Con A and E. coli bind to many surface glycoproteins (Fig. 2), internalization is mediated only by the 3G8 epitope-bearing Fc receptor. Several other studies proposing Con A receptors that cluster at the anterior pole of polarized neutrophils (30–32) are consistent with our model in that this location is identical with that described for Fc receptors in PMN oriented in a chemotactic gradient (31). Evidence for mannose residues on Fc receptors from murine peritoneal macrophages (33) and human monocytes (14), taken together with our findings, demonstrates that carbohydrate interactions allow classical immune system receptors in several cell types to participate in nonimmune phagocytosis of nonopsonized bacteria.

The importance of surface carbohydrates and surface lectins as recognition determinants in host defense against infection is well known (1). For example, lectin-like receptors for mannose and for advanced glycosylation endproducts on the surface of phagocytes mediate the binding and ingestion of such pathogens as *Leishmania donovani* and *Leishmania tropica major*, respectively (8, 9, 34). Even classical immune receptors, like the receptor for C3bi (CR3) on human monocytes, may have lectin-like properties (35). Alternatively, the bacterial surface may have lectin-like properties and bind to host cell surface carbohydrates (13). For example, *Mycoplasma pneumoniae* possess a surface protein that serves to bind the organisms to sialic acid residues (36, 37), *Vibrio cholerae* have surface lectins for fucose (38), and many Gram-negative bacteria, such as *E. coli*, *Salmonella*, and *Shigella*, have mannose-specific lectins (10–13, 39–41). In each instance the functional consequences of the binding of these microbes to host cells may be a reflection of the surface glycoprotein to which they adhere. The relationship between microorganism adhesion and pathogenicity or virulence has been described in many systems and may be particularly important in the initial stages of infection in a nonimmune host or in body sites where serum opsonins are deficient (42).

Since carbohydrate-lectin interactions are important in the pathogenesis of infectious diseases, heterogeneity in the expression of cell surface glycoproteins may contribute to differences in disease susceptibility among individuals. Genetic control of the susceptibility to bacterial infections has been described in murine and human studies (43, 44), but its exact mechanisms remain to be elucidated. Recent studies have demonstrated that there are genetically determined polymorphisms in Fc receptor expression and function on phagocytes (45–49). Some of these structural variations may be due to differences in the glycosylation of Fc receptors, analogous to those shown on human complement C3b receptors.
(50), and could influence the carbohydrate-lectin interactions between Fc receptors and microbes. Such Fc receptor polymorphisms among individuals might result in an association between genetically determined Fc receptor expression and the incidence of certain infections. Similarly, disease-associated alterations in Fc receptor structure or function may contribute to susceptibility to infection secondary to both opsonized and nonopsonized microbes.

Summary

We report that phagocytosis by human neutrophils of Con A–treated erythrocytes (E–Con A) and nonopsonized *Escherichia coli* with mannose-binding adhesions is mediated by the Fcγ receptor bearing the 3G8 epitope. Modulation of Fc receptors by pretreating with aggregated IgG or with 3G8 anti-Fcγ receptor mAb markedly inhibited internalization of E–Con A and *E. coli* without altering their cell surface attachment. Phagocytosis of these probes was specifically blocked by α-methylmannoside and D-mannose and not by other monosaccharides. Thus, recognition of E–Con A and *E. coli* by the Fc receptor is dependent upon the mannose-specific interaction with lectin or lectin-like adhesions. These data demonstrate that ligands other than the classical IgG opsonins can bind to classical immune receptors for IgG through lectin-carbohydrate interactions.

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