Monoclonal Antibody AHN-1 Inhibits Phagocytosis by Human Neutrophils

By Keith M. Skubitz, Daniel J. Weisdorf, and Phillip K. Peterson

The granulocyte-specific monoclonal antibody, AHN-1, immunoprecipitates two major surface-iodinated proteins of 105,000 and 145,000 to 150,000 daltons from normal human neutrophils. In this study, the effect of AHN-1 on a number of neutrophil functions was evaluated in vitro. Both complement- and antibody-mediated phagocytosis were inhibited when human neutrophils were pretreated with AHN-1 and opsonized bacteria were used as targets. The inhibition of phagocytosis was specific, in that lysosomal enzyme release and chemotaxis were not altered by treatment with AHN-1. AHN-1 did inhibit superoxide production by neutrophils in response to particulate stimuli, but not in response to the soluble stimulus, 12-O-tetradecanoylphorbol-13-acetate. The data indicate that one or both of these surface proteins may be important in the process of phagocytosis. AHN-1 should be useful in isolating and further characterizing the nature of these molecules.

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THE NEUTROPHIL PLAYS a central role in host defense and is a major effector cell that causes tissue damage in a variety of disorders. While the molecular basis for these effects is not well established, glycoproteins of the plasma membrane are believed to play a prominent role in many of the neutrophil's functions. Changes in the plasma membrane composition of the neutrophil have been observed following the cell's response to stimulation.1,2 Defects in neutrophil function, with concomitant increased susceptibility to infections, have been associated with a deficiency of a 180,000-dalton glycoprotein of the neutrophil plasma membrane in three patients3,4 and with a deficiency of a different glycoprotein of 150,000 daltons in another.5

Studies of plasma membrane proteins of neutrophils have been hindered by difficulty in obtaining membrane preparations of high purity and in identifying and purifying specific membrane proteins. Monoclonal antibodies to human neutrophil antigens may help solve these problems. An antineutrophil monoclonal antibody has been described that blocks lysosomal enzyme release and chemotaxis, but not phagocytosis.6 Another monoclonal antibody has been reported that inhibits N-Formyl-Met-Leu-Phe (fMLP)-induced chemotaxis of human neutrophils,7 but not the binding of fMLP to its surface receptor.7 The nature of the antigen in each case remains undefined.

Two monoclonal antibodies to the Mo1 granulocyte-monocyte antigen have recently been reported to inhibit phagocytosis of complement (C3) or IgG-coated particles by neutrophils.8,9 These antibodies also inhibited rosetting between phagocytes and erythrocytes coated with C3bi and inhibited lysosomal enzyme release from granulocytes stimulated with zymosan that was coated with C3bi. Both antibodies immunoprecipitated two proteins of 155,000 and 94,000 daltons from neutrophils surface-labeled with 125I. The PMN7C3 antibody has recently been shown to inhibit zymosan activation of neutrophil oxidative metabolism.10 The exact nature of the epitopes recognized by these antibodies is not yet known.

We have studied the effects of the anti-human neutrophil monoclonal antibody, AHN-1, on neutrophil function in vitro. We have previously shown that this monoclonal antibody recognizes an oligosaccharide that is present on two major surface-iodinated proteins of human neutrophils.11-14 AHN-1 selectively blocked phagocytosis of opsonized bacteria. AHN-1 preferentially inhibited complement-mediated phagocytosis, but had less effect on Fc receptor-mediated phagocytosis. Lysosomal enzyme release and chemotaxis were unaffected by AHN-1. AHN-1 inhibited superoxide production by neutrophils in response to particulate stimuli, but not in response to the soluble stimulus 12-O-tetradecanoylphorbol-13-acetate (PMA). These results suggest that one or both of the surface proteins recognized by AHN-1 may be important in phagocytosis. The functional neutrophil defect induced by this antibody resembles that described in neutrophils genetically deficient in a 150,000-dalton membrane protein.5

Materials and Methods

Antibodies

Anti-human neutrophil monoclonal antibody AHN-1 was produced as previously described. Eight-week-old female C57BL/6J mice (Jackson Laboratories, Bar Harbor, Me) were immunized by intraperitoneal injection of 10⁷ neutrophils in Freund's complete adjuvant (GIBCO Laboratories, Grand Island, NY), and subsequently, at weekly intervals, intraperitoneally with 10⁶ neutrophils in phosphate-buffered saline (PBS) and subcutaneously with 10⁷ neutrophils in incomplete Freund's adjuvant. One week following the third immunization, mice received 10⁷ neutrophils in PBS intraperitoneally. Three days later, their spleen cells were fused with murine myeloma P3-X63-Ag8.653 cells at a ratio of five spleen cells per myeloma cell. Hybridoma cell cultures secreting antibodies that bound to neutrophils were twice cloned in soft agar. Ascites fluid from a mouse bearing the IgM-secreting hybridoma AHN-1 was heat-inactivated at 56°C for 30 minutes, concentrated by centrifugation at 100,000 g for one hour. Antibody purity was routinely revealed greater than 95% neutrophils.

Cell Preparation

Normal peripheral blood neutrophils were prepared by a modification of the method of Böyum, as previously described. Cells were suspended at appropriate concentrations in a buffer solution, pH 7.2, consisting of 135 mmol/L NaCl, 4.5 mmol/L KCl, 1.3 g/L glucose, and 10 mmol/L Na₂H₂PO₄·H₂O, and 10 latex beads (polystyrene microspheres, 0.91 μm diameter, Polysciences Inc, Warrington, Pa) were then added, and the mixture was incubated for 30 minutes at 37°C with gentle shaking. Phagocytosis was terminated by adding 1 mL of ice-cold HBSS and centrifuging at 400 g for five minutes. Cytosin preparations were prepared, fixed with methanol for five minutes, stained with Fast Green FCF (C.I. 42053) (Eastman Kodak, Rochester, NY) (0.01% in water. 15 seconds), and examined by light microscopy. Results were expressed as percent of cells containing one or more completely ingested beads.

To further evaluate nonspecific (opsonin-independent) phagocytosis, a recently described assay was used in which neutrophils were added to plastic tissue culture wells containing adherent bacteria, and phagocytosis of these bacteria was determined exactly as described.

Chemotaxis

Cell migration was assayed in modified Boyden chambers using mixed celluloace and nitrate filters of 3.0 μm mean pore size and thickness of 158 ± 16 μm (Milipore Corp, Bedford, Mass) by the leading front technique after migration for 60 minutes at 37°C. Neutrophils (10⁶ in 100 μL of HBSS-HSA) were mixed with 100 μL of HBSS containing 0.5% HSA in the lower chamber, and the mixtures were incubated for 15 minutes at 37°C. The cells were then placed in the upper chamber of a prewarmed 37°C Boyden apparatus and incubated at 37°C for 60 minutes. Random migration was assayed using HBSS, containing 0.5% HSA in the lower chamber, and chemotaxis was measured using 10² mol/L FMLP (Sigma Chemical Co, St Louis) in the lower chamber. The concentration of chemotactic stimulus was chosen because it normally induces intermediate rates of migration. Chemokinesis was tested by adding equal concentrations of antibody AHN-1 to the upper and lower chambers. Migration distance was determined microscopically by quintuplicate readings of triplicate coded filters.

Superoxide Generation

Superoxide generation was assayed by quantitating the reduction of cytochrome C in the presence and absence of superoxide dismutase, as previously described. Neutrophils (10⁶ in 100 μL of HBSS) were mixed with 100 μL of GHBSS containing twice the desired final concentration of antibody, and the mixture was incubated for 15 minutes at 37°C. Cytochrome C (Sigma) (final concentration 100 μmol/L), superoxide dismutase (Sigma) (final concentration 40 μg/mL), or HEPES-buffered saline, and stimuli (PMA, Sigma; final concentration 10 ng/mL) or 500 μg/mL (final concentration) of zymosan (ICN Pharmaceuticals, Cincinnati) oposened with fresh human serum (2 μg/mL) for 30 minutes at 37°C, or serum oposened E. coli strain 011B (3 × 10⁶ CFU/mL) final concentration) were then added to a total incubation volume of 500 μL, and the mixtures were incubated for 15 minutes or 30 minutes at 37°C. The reaction was terminated by adding 500 μL of ice-cold HBSS.
centrifuging at 12,000 g for five minutes, and 900 µL of supernatant was removed. The absorbance (550 nm) of the supernatant fluids were assayed in quadruplicate before and after the addition of potassium ferricyanide. Superoxide generation was expressed as nmol cytochrome C reduced/10⁶ neutrophils/30 min.

Degranulation

Neutrophil degranulation was assayed by measuring the extracellular release of cytoplasmic and granule enzymes, as previously described. Briefly, 5 x 10⁶ neutrophils in 50 µL of HEPES-buffered saline was mixed with 50 µL of GHBSS containing the appropriate dilution of antibody to yield the desired final antibody concentration, and the mixture was incubated for 15 minutes at 23 °C. The mixture was then warmed to 37 °C, and 100 µL of the following reagents (prewarmed to 37 °C) as appropriate were added: HBSS-HSA, opsonized zymosan (500 µg/mL final concentration), fMLP (10⁻⁷ mol/L final concentration), and cytochalasin B (5 µg/mL final concentration). The mixtures were then incubated for 15 or 30 minutes at 37 °C with shaking. The reaction was terminated by adding 800 µL of ice-cold HBSS and centrifuging at 12,000 g for five minutes. Enzyme activities in the supernatants were assayed by standard methods. Lysozyme was measured by use of a commercially prepared kit (Worthington Biochemical Corp, Freehold, NJ), as previously described. β-Glucuronidase was assayed by a modification of the technique of Fishman and myeloperoxidase by the technique of Lundquist and Josefsson. Lactate dehydrogenase was measured by a modification of the method of Beutler.

Statistics

Inhibition of neutrophil function by various dilutions of antibody was analyzed using Jonckheere’s test or an unpaired Student’s t test.

Solid Phase Binding Assay

Antibody binding to detergent extracts or glycolipid preparations from normal human neutrophils was tested using a solid-phase binding assay as previously described. Briefly, detergent extracts were prepared by solubilizing neutrophils in a buffer solution containing 5 mmol/L Tris-HCl (pH 9.2), 1 mmol/L EDTA, 400 mmol/L KCl, 1% Triton X-100, and 2 mmol/L phenyl-methyl-sulfonyl-fluoride as described; glycolipid preparations were prepared according to the method of Svennerholm and Fredman as described. These neutrophil extracts were then diluted to various concentrations, and 50-µL aliquots were applied to wells of Linbro 96-well microtiter plates (Flow Laboratories, Inc, Hamden, Conn) and dried overnight at 23 °C. Two hundred microliters of PBS containing 50 mg/mL BSA and 0.02% NaN₃ was then added to each well and incubated four hours at 23 °C. This solution was removed and antibody binding was tested as described using ¹²⁵I-labeled goat anti-mouse immunoglobulin (Amersham, Arlington Heights, Ill).

Live Cell Binding Assay

Binding of AHN-1 to the surface of live human neutrophils was quantitated exactly as previously described.

RESULTS

Phagocytosis

Phagocytosis of S. epidermidis opsonized with purified IgG was tested to measure Fc receptor-mediated phagocytosis. Phagocytosis of E. coli preopsonized with pooled human serum was used as a measure of C3 receptor-mediated phagocytosis. Phagocytosis of IgG-opsonized (2 mg/mL IgG) S. epidermidis by control (untreated) neutrophils was 54.3% ± 2.7% (SEM), while that of E. coli opsonized with pooled human serum (20% serum) was 52.7% ± 2.1% (SEM). Heat inactivation (56 °C for 30 minutes) of pooled human serum abolished its opsonic activity for E. coli. When unopsonized E. coli or S. epidermidis was tested, 10% of the target bacteria were phagocytosed.

Antibody AHN-1 inhibited, in a dose-dependent manner, neutrophil phagocytosis of opsonized bacteria (Fig 1). AHN-1 was more potent at inhibiting phagocytosis of E. coli (67% inhibition at an antibody concentration of 20 µg/mL) than S. epidermidis (25% inhibition at 20 µg AHN-1/mL). The inhibition of phagocytosis by AHN-1 was highly statistically significant (P < .005 by Jonckheere’s test for both target bacteria). The inhibition of phagocytosis by AHN-1 correlated well with determinations of antibody binding to live human neutrophils. Antibody binding to live neutrophils plateaued at an AHN-1 concentration of 20 to 40 µg/mL; similarly, the inhibitory effect on phagocytosis was nearly maximal at an AHN-1 concentration of ~20 µg/mL. Four control antineutrophil monoclonal antibodies had no effect on phagocytosis at concentrations up to 40 µg/mL: phagocytosis of E. coli and S. epidermidis was 120% and 95% of control, respectively, in the presence of antibody 208; 100% and 97% of control, respectively, in the presence of antibody 54; 100% and 99% of control, respectively, in the presence of AHN-8, and 100% and 98% of control, respectively, in the presence of antibody 238. Light microscopic studies of Wright-stained cytocentrifuge preparations confirmed that AHN-1 inhibited phago-
cytosis of the target bacteria (data not shown). As expected, no binding of AHN-1 to either opsonized bacteria could be detected (data not shown).

Since AHN-1 was more potent at inhibiting phagocytosis of E coli than S epidermidis (Fig 1), we tested whether this could be due to the concentrations of opsonins on the surface of the target bacteria. Varying the concentration of IgG used to opsonize the S epidermidis from 2.0 to 0.02 mg/mL, decreased the phagocytosis by control cells but did not influence the amount of inhibition induced by 10 μg/mL AHN-1 (data not shown). The effect of AHN-1 on two opsonin-independent phagocytosis systems was also tested. AHN-1 (10 μg/mL) had no effect on phagocytosis of latex beads or phagocytosis of surface-adherent unopsonized S epidermidis ST (data not shown).

Chemotaxis

Neither antibody AHN-1 (Table 1) nor the control antibody AHN-8 (data not shown) at any concentration tested had any effect on neutrophil chemotaxis toward fMLP. Similarly, AHN-1 had no effect on random migration (Table 1) or chemokinesis (data not shown).

Superoxide Generation

Neither antibody AHN-1 (Fig 2) nor the control antibody AHN-8 (data not shown) inhibited the production of superoxide by resting neutrophils or cells stimulated with PMA. AHN-1 (Fig 2), but not AHN-8 (not shown), had a small inhibitory effect on the production of superoxide by neutrophils stimulated with opsonized zymosan. As expected, AHN-1 did not bind to opsonized zymosan (data not shown). Since zymosan is a stronger stimulus than bacteria, we also tested the effect of AHN-1 on superoxide production by neutrophils stimulated with opsonized E coli ON2 (Table 2). Opsonized E coli was a weak stimulus. Using a particle-neutrophil ratio of 300:1 and a 30-minute incubation, we were able to stimulate measurable superoxide production. At this bacteria-neutrophil ratio, AHN-1 had no effect on superoxide production (Table 2). Similar experiments using a 15-minute stimulation with either PMA (Table 2) or opsonized E coli (data not shown) also showed no effect of AHN-1 on superoxide production.

Degranulation

Neither AHN-1 (Table 3) nor the control antibody AHN-8 (data not shown) had any statistically significant effect on specific granule release by resting or stimulated neutrophils. Superoxide production was assayed as nmol cytochrome C reduced/10^6 neutrophils/30 min. At this bacterium-neutrophil ratio, AHN-1 had no effect on superoxide production (Table 2). Similar experiments using a 15-minute stimulation with either PMA (Table 2) or opsonized E coli (data not shown) also showed no effect of AHN-1 on superoxide production.

Table 1. Effect of AHN-1 on Neutrophil Chemotaxis

<table>
<thead>
<tr>
<th>Antibody Concentration (μg/mL)</th>
<th>Migration (μm/h)</th>
<th>Random Migration</th>
<th>Chemotaxis to fMLP</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>32.0 ± 9.5</td>
<td>111.0 ± 9.0</td>
<td></td>
</tr>
<tr>
<td>0.8</td>
<td>23.7 ± 2.9</td>
<td>123.7 ± 29.3</td>
<td></td>
</tr>
<tr>
<td>4.0</td>
<td>27.3 ± 2.1</td>
<td>96.7 ± 19.4</td>
<td></td>
</tr>
<tr>
<td>20.0</td>
<td>30.3 ± 11.0</td>
<td>101.0 ± 9.0</td>
<td></td>
</tr>
</tbody>
</table>

Antibody AHN-1 had no effect on neutrophil random migration or chemotaxis response to fMLP. Chemotaxis was assayed as described in the text. Migration (reported as μm/h) is shown when the lower chamber contained buffer (random migration) or 10^8 mol/L fMLP (chemotaxis), for various dilutions of antibody, as indicated. Each value is the mean ± SD of filters from three separate chambers. A duplicate experiment gave similar results.

Table 2. Effect of AHN-1 on Neutrophil Superoxide Production (nmol cytochrome C reduced/10^6 neutrophils/30 min)*

<table>
<thead>
<tr>
<th>Antibody Concentration (μg/mL)</th>
<th>Stimulus</th>
<th>E coli ON2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Buffer</td>
<td>PMA†</td>
</tr>
<tr>
<td>0.0</td>
<td>0.6 ± 0.1</td>
<td>23.7 ± 1.2</td>
</tr>
<tr>
<td>0.8</td>
<td>0.5 ± 0.3</td>
<td>21.9 ± 1.3</td>
</tr>
<tr>
<td>4.0</td>
<td>0.6 ± 0.2</td>
<td>21.4 ± 1.8</td>
</tr>
<tr>
<td>20.0</td>
<td>1.0 ± 0.9</td>
<td>20.8 ± 2.2</td>
</tr>
</tbody>
</table>

*Antibody AHN-1 had no effect on the production of superoxide by resting or stimulated neutrophils. Superoxide production was assayed as described in the text. Neutrophils were preincubated with antibody for 15 minutes at 23 °C prior to adding the stimuli (HEPES-buffered saline, 10 ng/mL PMA, or 3 × 10^8 CFU/mL serum-opsonized E coli ON2). The amount of superoxide produced by 10^6 neutrophils is shown in nmol cytochrome C reduced in 30 minutes at 37 °C for each final concentration of antibody and each stimulus. Each value is the mean of four separate determinations ± SD. A duplicate experiment gave similar results.

†Superoxide production by PMA-stimulated cells is shown as nmol cytochrome C reduced/10^6 neutrophils/15 min.
ANTIBODY AHN-1 INHIBITS PHAGOCYTOSIS

Cells were stimulated by 10^{-7} M fMLP (fMLP) in the presence of 5 μg/mL cytochalasin B (CB) or with 500 μg/mL opsonized zymosan. Each enzyme release, as described in the text. The quantity of each enzyme released per 5 × 10^6 cells is shown as a percent of the total enzyme content of the cells. Two additional duplicate experiments gave similar results.

Antibody Binding to Cell Extracts

Antibody AHN-1 had little effect on neutrophil degranulation. Neutrophils were pretreated with the final dilution of antibody shown and assayed for enzyme release, as described in the text. The quantity of each enzyme released per 5 × 10^6 cells is shown as a percent of the total enzyme content of the cells. Cells were stimulated by 10^{-7} mol/L fMLP (fMLP) in the presence of 5 μg/mL cytochalasin B (CB) or with 500 μg/mL opsonized zymosan. Each value is the mean ± SD of triplicate samples. Analysis by a Student’s t test failed to demonstrate any statistically significant (P < .05) differences between AHN-1 and buffer-treated cells. Two additional duplicate experiments gave similar results.

Table 3. Effect of AHN-1 on Neutrophil Enzyme Release (Percentage of Release)

<table>
<thead>
<tr>
<th>Antibody Concentration (μg/mL)</th>
<th>Enzyme</th>
<th>Myeloperoxidase</th>
<th>β-Glucuronidase</th>
<th>Lysozyme</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer</td>
<td></td>
<td>2.0 ± 2.0</td>
<td>6.8 ± 1.1</td>
<td>5.4 ± 2.3</td>
</tr>
<tr>
<td>CB</td>
<td></td>
<td>1.9 ± 2.1</td>
<td>7.5 ± 1.1</td>
<td>4.3 ± 0.8</td>
</tr>
<tr>
<td>CB + fMLP</td>
<td></td>
<td>2.0 ± 1.0</td>
<td>6.8 ± 1.1</td>
<td>2.1 ± 0.8</td>
</tr>
<tr>
<td>Opsonized zymosan</td>
<td></td>
<td>8.4 ± 2.7</td>
<td>19.3 ± 4.5</td>
<td>25.7 ± 6.4</td>
</tr>
</tbody>
</table>

Antibody AHN-1 had little effect on neutrophil degranulation. Neutrophils were pretreated with the final dilution of antibody shown and assayed for enzyme release, as described in the text. The quantity of each enzyme released per 5 × 10^6 cells is shown as a percent of the total enzyme content of the cells. Cells were stimulated by 10^{-7} mol/L fMLP (fMLP) in the presence of 5 μg/mL cytochalasin B (CB) or with 500 μg/mL opsonized zymosan. Each value is the mean ± SD of triplicate samples. Analysis by a Student’s t test failed to demonstrate any statistically significant (P < .05) differences between AHN-1 and buffer-treated cells. Two additional duplicate experiments gave similar results.

Antibody Binding to Cell Extracts

AHN-1 is known to bind a carbohydrate epitope present on neutrophil glycolipids as well as the 105 and 145 kd proteins.12-14 We therefore quantitated the relative amounts of antigen present in a neutrophil glycolipid extract in comparison with that present in a detergent extract of neutrophils. Detergent extracts and glycolipid extracts were prepared from equal numbers of normal human neutrophils as described in Materials and Methods. The volumes of these extracts were then equalized and aliquots of serial dilutions of these extracts were used as target antigen in the solid-phase binding assay. AHN-1 binding could readily be detected in wells containing detergent extracts at target antigen concentrations at least 500-fold more dilute than in wells containing glycolipid extract (data not shown). The glycolipid preparation used here has been reported to extract the vast majority of glycolipids present in cells.33 Furthermore, glycolipids are known to bind avidly to the microtiter wells under the conditions used here (C. Blackburn and R. Schnarr, The Johns Hopkins University School of Medicine, unpublished observation, 1982). Therefore, these results suggest that in neutrophils, only a small percentage of antigen recognized by AHN-1 is present on glycolipids.

DISCUSSION

Plasma membrane glycoproteins are believed to have an important role in many of the diverse functions of the neutrophil. We recently described the granulocyte-specific monoclonal antibody AHN-1.11 AHN-1 reacts specifically with human neutrophils among peripheral blood cells, having no reactivity with red blood cells, lymphocytes, monocytes, basophils, or platelets.11 This antibody has been shown to recognize an oligosaccharide present on two major surface-iiodinated proteins of human neutrophils and on neutrophil glycolipids.12 14 We report that AHN-1 selectively blocked phagocytosis of opsonized bacteria by human neutrophils, while a control monoclonal antibody did not. AHN-1 was more potent in inhibiting the phagocytosis of opsonized E coli ON2, which is mediated by C3 receptors,20-22 than that of opsonized S epidermidis, which is mediated primarily by Fc receptors.20-22 Despite the marked effect of AHN-1 on phagocytosis, the antibody had no effect on either chemotaxis or granule release. AHN-1 did have a slight inhibitory effect on superoxide production by neutrophils stimulated with opsonized zymosan, but not by cells stimulated with the soluble stimulus PMA. Thus, the effect of AHN-1 on neutrophil function was quite specific. We have previously shown that AHN-1 reacts with a carbohydrate epitope present on neutrophil glycolipids as well as proteins.12-14 The results of the solid-phase binding assay suggested that in neutrophils, the great majority of antigen recognized by AHN-1 was present on glycoproteins, while a much
smaller, yet still detectable, quantity was present on glycolipids. These results suggest that one or both of the surface glycoproteins that bind AHN-1 are involved in the process of phagocytosis. Neutrophils are known to have distinct surface Fc and C3b receptors, and these have been partially characterized.34-36 One possible site of action of AHN-1 in inhibiting phagocytosis could be at the level of these receptors. However, neither of the proteins recognized by AHN-111,12 resemble the neutrophil Fc or C3b receptors previously described. Further studies will be necessary to determine whether AHN-1 exerts its effect by inhibiting target attachment, internalization, or both.

Other monoclonal antineutrophil antibodies have been described that affect neutrophil function. One antibody, NCD1, blocks lysosomal enzyme release and chemotaxis, but not phagocytosis.9 Another antibody, NCD3, inhibits chemotaxis induced by the synthetic chemotaxin, N-Formyl-Met-Leu-Phe,7 although it does not interfere with binding of the chemotaxin to its receptor. The biochemical nature of the antigens recognized by these antibodies remains unknown. The antigranulocyte antibody WEM-G1 stimulates neutrophil function as measured by antibody-dependent cell-mediated cytotoxicity assays.37 The 60.3 antibody, which reacts with most peripheral blood leukocytes, immunoprecipitates proteins of similar molecular weight to those recognized by AHN-1 and inhibits neutrophil chemotaxis.38,39 Monoclonal antibodies against the Mol antigen have also recently been reported to inhibit phagocytosis of particles opsonized with either C3 or IgG.8,9 These antibodies, in contrast to AHN-1, react with both neutrophils and monocytes; however, they immunoprecipitate two proteins of 155,000 and 94,000 daltons from neutrophils surface-labeled with 125I, which are similar to those recognized by AHN-1. The proteins immunoprecipitated by anti-Mol are structurally and antigenically related to the lymphocyte function-associated antigen.40 In addition, the PMN7C3 antibody has been shown to inhibit zymosan activation of neutrophil oxidative metabolism.10 The exact nature of the epitopes recognized by these antibodies is unknown.

Defects in neutrophil function have been associated with deficiencies of membrane proteins.3-3,38,41 Neutrophils from one patient lacked a major surface-iodinated membrane protein of 150,000 mol wt and also exhibited impaired phagocytosis.7 Despite the phagocytic defect, various other neutrophil functions were normal, including superoxide production in response to soluble stimuli, lysosomal enzyme release, and chemotaxis. A similar patient with neutrophil dysfunction has been described whose neutrophils lack the Lp95-150 antigen complex recognized by 60.3.39 The similarity of the functional defect induced in normal neutrophils by treatment with AHN-1 to that reported by Arnaout et al.10 in neutrophils genetically deficient in a major surface-iodinated membrane protein of 150,000 mol wt suggests that AHN-1 may impair phagocytosis by interacting with the 145 to 150 kd protein and that this protein may be the same protein that is deficient in neutrophils of this patient. It remains unknown, however, whether the inhibition of phagocytosis seen in neutrophils treated with AHN-1 is solely the result of the interaction of the antibody with the 145 kd protein, the 105 kd protein, or another molecule. This antibody should be useful in purifying these proteins, as well as studying their roles in neutrophil function.

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