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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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 10⁷ 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, and then ammonium sulfate fractionation, and dialyzed against PBS, pH 7.4. The ammonium sulfate fractionation was repeated, and the antibody-containing fraction was dialyzed against PBS overnight at 4 °C. Further purification of AHN-1 was obtained by Sephacryl S-300 (Pharmacia Fine Chemicals, Piscataway, NJ) molecular sieve chromatography. Prior to use, the antibody solution was clarified by centrifugation at 100,000 g for one hour. Antibody purity was assessed by NaDodSO₄ polyacrylamide gel electrophoresis (SDS-PAGE) and Coomassie brilliant blue staining. Antibody concentration was estimated spectrophotometrically (A₂₈₀). Serial dilutions of the antibody preparation (4 mg/mL) were made in Hanks' balanced salt solution (HBSS; GIBCO). Ascites fluid from mouse bearing hybridomas AHN-1, AHN-2 (IgGl), and three other hybridomas, 54 (IgGl), 238 (IgM), and 208 (IgM) were used as control antigranulocyte antibodies. Each of these antibodies binds to the surface of live human 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 N₂H₂-hydroxyethylpiperazine-N'₂-ethanesulfonic acid (HEPES-buffered saline) or HBSS containing 0.2% human serum albumin (Cutter Laboratories, Berkeley, Calif) (HBSS-HSA). Differential cell counts performed on Wright-stained cells routinely revealed greater than 95% neutrophils.

Phagocytosis

Phagocytosis of opsonized ³H-thymidine-labeled bacteria was tested as previously described. Staphylococcus epidermidis strain ST (5 × 10⁷ colony-forming units [CFU]), incubated for 30 minutes at 37 °C with 2 mg/mL purified human IgG (Hyland Laboratories, Glendale, Calif) at the concentrations indicated, and Escherichia coli strain ON2 (5 × 10⁷ CFU), opsonized for 30 minutes at 37 °C with pooled human serum at the concentrations indicated, were used as targets. In this system, phagocytosis of E coli and S epidermidis has been shown to be primarily dependent on C3 and Fc receptors, respectively. Briefly, 5 × 10⁷ neutrophils in 50 μL of HBSS containing 0.5% HSA were mixed with 50 μL of AHN-1 antibody or control antibody diluted in HBSS. The cells were incubated 15 minutes at 37 °C with the antibody at the concentration indicated. One hundred microliters of preopsonized bacteria (5 × 10⁷ CFU in HBSS) were then added to polystyrene microslides (Bio-slides, Beckman Instruments, Fullerton, Calif) containing 5 × 10⁷ neutrophils, and the mixtures were incubated at 37 °C for 30 minutes with shaking. Phagocytosis was stopped by adding 3 mL of ice-cold PBS, and non–cell-associated bacteria were removed by three cycles of differential centrifugation. Phagocytosis was expressed as the percentage of uptake of total added radioactivity, which was determined in a separate vial.

Light microscopic studies of Wright-stained cytocentrifuge preparations were used as a morphological confirmation of phagocytosis.

Phagocytosis of latex particles was tested as follows: 50 μL of HBSS-HSA containing 5 × 10⁷ neutrophils was mixed with 50 μL of antibody AHN-1 or control antibody diluted in GHBSS, and the mixture was incubated for 15 minutes at 23 °C. Fifty microliters of HBSS containing 0.5% HSA and 10³ latex beads (poly styrene 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.1. 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 cellucrose acetate and nitrate filters of 3.0 μm mean pore size and thickness of 158 ± 16 μm (Millipore 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 GHBSS that contained antibody AHN-1 or control antibody (final concentration as indicated), and the mixtures were incubated for 15 minutes at 23 °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 10⁻⁵ 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 23 °C. Cytochrome C (Sigma) (final concentration 200 μg/mL), 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) opsonized with fresh human serum (2 mg/mL) for 30 minutes at 37 °C, or serum opsonized E coli strain ON2 (3 × 10⁷ 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 cytospin 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

### Table 1. Effect of AHN-1 on Neutrophil Chemotaxis

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

Antibody AHN-1 had no effect on neutrophil random migration or chemotaxis in 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/10⁶ neutrophils/30 min)*

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Concentration (μg/mL)</th>
<th>Buffer</th>
<th>PMA†</th>
<th>*E coli ON2</th>
</tr>
</thead>
<tbody>
<tr>
<td>AHN-1</td>
<td>0.6 ± 0.1</td>
<td>23.7 ± 1.2</td>
<td>1.2 ± 0.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.8 ± 0.3</td>
<td>21.9 ± 1.3</td>
<td>1.1 ± 0.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20.0 ± 0.2</td>
<td>21.4 ± 1.8</td>
<td>1.4 ± 0.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20.0 ± 0.9</td>
<td>20.8 ± 2.2</td>
<td>1.3 ± 0.4</td>
<td></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 x 10⁹ CFU/mL serum-opsonized *E coli ON2*). The amount of superoxide produced by 10⁶ 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/10⁶ neutrophils/15 min.
ANTIBODY AHN-1 INHIBITS PHAGOCYTOSIS

The quantity of each enzyme released per 5 x 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 ng/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>Stimulus</th>
<th>Antibody Concentration (μg/mL)</th>
<th>Enzyme</th>
<th>Myeloperoxidase</th>
<th>β-Glucuronidase</th>
<th>Lysozyme</th>
</tr>
</thead>
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<tr>
<td>Buffer</td>
<td>0</td>
<td></td>
<td>2.0 ± 2.0</td>
<td>6.8 ± 1.1</td>
<td>5.4 ± 2.3</td>
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<tr>
<td></td>
<td>20</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</td>
<td>0</td>
<td></td>
<td>2.0 ± 1.0</td>
<td>6.8 ± 1.1</td>
<td>2.1 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td></td>
<td>0.6 ± 0.6</td>
<td>7.9 ± 1.1</td>
<td>2.1 ± 0.8</td>
</tr>
<tr>
<td>CB + fMLP</td>
<td>0</td>
<td></td>
<td>11.7 ± 5.0</td>
<td>23.9 ± 10.2</td>
<td>30.7 ± 8.4</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td></td>
<td>8.4 ± 2.7</td>
<td>19.3 ± 4.5</td>
<td>25.7 ± 6.4</td>
</tr>
<tr>
<td>Opsonized zymosan</td>
<td>0</td>
<td></td>
<td>2.7 ± 0.8</td>
<td>15.9 ± 1.1</td>
<td>16.1 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td></td>
<td>2.8 ± 0.4</td>
<td>14.8 ± 1.1</td>
<td>12.9 ± 1.4</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 x 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.

Stimulated neutrophils, as measured by release of the secondary granule enzyme lysozyme. Similarly, neither AHN-1 (Table 3) nor AHN-8 (data not shown) inhibited release of the primary granule enzymes, β-glucuronidase, or myeloperoxidase from resting or stimulated cells. Furthermore, neither antibody exhibited any cytotoxic effect on neutrophils in these experiments, as determined by measuring the release of cytoplasmic lactate dehydrogenase (data not shown). At higher concentrations of stimuli, AHN-1 also had no effect on enzyme release (data not shown). We also wished to study the effect of AHN-1 on enzyme release from neutrophils stimulated with opsonized bacteria; however, we were unable to induce measurable specific enzyme release using opsonized E. coli ON2 as a stimulus, even using particle-neutrophil ratios as high as 1,000:1.

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. 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. 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. AHN-1 reacts specifically with human neutrophils among peripheral blood cells, having no reactivity with red blood cells, lymphocytes, monocytes, basophils, or platelets. This antibody has been shown to recognize an oligosaccharide present on two major surface-iodinated proteins of human neutrophils and on neutrophil glycolipids. 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, than that of opsonized S. epidermidis, which is mediated primarily by Fc receptors. 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. 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. 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-1 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. Another antibody, NCD3, inhibits chemotaxis induced by the synthetic chemotaxin, N-Formyl-Met-Leu-Phe, 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. 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. Monoclonal antibodies against the Mol antigen have also recently been reported to inhibit phagocytosis of particles opsonized with either C3 or IgG. 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. In addition, the PMN7C3 antibody has been shown to inhibit zymosan activation of neutrophil oxidative metabolism. The exact nature of the epitopes recognized by these antibodies is unknown.

Defects in neutrophil function have been associated with deficiencies of membrane proteins. Neutrophils from one patient lacked a major surface-iodinated membrane protein of 150,000 mol wt and also exhibited impaired phagocytosis. 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. The similarity of the functional defect induced in normal neutrophils by treatment with AHN-1 to that reported by Arnaout et al 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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