Identification of the Major Lectin-Binding Surface Proteins of Human Neutrophils and Alveolar Macrophages

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Concanavalin A (Con A) and wheat germ agglutinin (WGA) are frequently used as stimuli of neutrophils and macrophages. While the effects of these lectins on cell function are presumably mediated by interaction with cell-surface molecules, the target structures on the cell surface involved are not well defined. We have used the techniques of lactoperoxidase catalyzed cell-surface iodination, lectin affinity chromatography, monoclonal antibody immunoprecipitation, and NaDodSO4-polyacrylamide gel electrophoresis to study the surface proteins of human neutrophils and alveolar macrophages that react with six lectins including Con A and WGA. We found that several major surface-labeled proteins of neutrophils bound Con A. Four of these proteins were identified by immunoprecipitation as members of the LFA-1/HMac-1/gp150,95 adhesion glycoprotein family. Con A also bound CR1 and a 135-kd surface-labeled protein recognized by CD15 monoclonal antibodies. WGA also bound many of these proteins, but had a much lower avidity for CR1. All three of the major surface-labeled proteins of human alveolar macrophages bound to Con A, including the 183-kd mannose receptor and the 30-kd smoking-associated protein. WGA also bound the 183-kd macrophage protein, but not the 30-kd protein. These results should aid the understanding of studies using these lectins as stimuli.

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METHODS

Materials. Lectins bound to agarose beads (DBA, PNA, RCA, UEA, WGA) and L-Fucose were obtained from E-Y Labs, Inc, San Mateo, CA. The Con A-Sepharose was obtained from Pharmacia Inc, Piscataway, NJ. Methyl-α-D-mannopyranoside, N-acetyl-galactosamine, D-galactose, and N-acetyl-glucosamine were obtained from the Sigma Chemical Company, St Louis. Monoclonal antibodies AHN-1 (IgM) and OKM1 (IgG2b) have been previously described.23 Monoclonal antibody YZ-1 (IgG1)26 was a gift from Dr M. Hostetter, University of Minnesota Medical School, Minneapolis, and antibody 60.3 (IgG2a)27 was a gift from Drs J. Harlan and P. Beatty, University of Washington Medical School, Seattle.

Cell preparation. Normal peripheral blood neutrophils were prepared by a modification of the method of Boyum as previously described24 and were suspended at the indicated concentrations in phosphate-buffered saline (PBS), pH 7.4. Differential cell counts on Wright-stained cells routinely revealed >95% neutrophils.

In some cases, as indicated, neutrophils were also obtained by leukapheresis. The cells were obtained in a final volume of 200 mL containing 16 mL of Tris-sodium citrate with 6% hydroxyethyl starch. Portions of the leukapheresis preparations were further purified by dextran sedimentation and hypotonic lysis as described above except that the Ficoll-Paque step was eliminated. Cells were then washed four times with PBS before being solubilized as described above.

Alveolar macrophages were obtained from Drs J. Hoidal, University of Tennessee Medical School, Memphis, and S. McGowan, University of Minnesota Medical School. Bronchoscopic sterile
saline lavage of the lingula of the left lung or the right middle lobe was done in healthy smoking (minimum one-pack-per-day smokers) volunteers. All patients were advised of procedures and attendant risks, in accordance with institutional guidelines, and gave informed consent. Volumes of lavage fluid instilled and returned were comparable in each subject. Recovered cells were separated from lavage fluid by centrifugation at 400 g for ten minutes at 23°C, washed three times with PBS, and resuspended in PBS. Cells were characterized by staining cytosin slide preparations with Wright's stain or nonspecific esterase stain counterstained with Mayer's hematoxylin. Examination of Wright's-stained slides confirmed that >95% of the cells were esterase positive. Viability of alveolar macrophages was >90%.

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**RESULTS**

**"I-labeled neutrophils.** Surface proteins of neutrophils labeled with "I by the lactoperoxidase technique were fractionated by lectin affinity chromatography and SDS-PAGE. Differences in protein binding patterns were observed among the six lectin affinity columns (Fig 1). The major proteins present in the cell extract before affinity chromatography included those of 200-, 185-, 135-, 115-, and a broad band from 72- to 82-kd (lane A). There was no enrichment of "I-labeled proteins by chromatography on plain agarose beads (lane B), PNA (lane G), or DBA (lane H). The composition of "I-labeled proteins present in the run throughs from these columns (lanes I, N, and O) was similar to that of the initial cell extract applied to the column (lane A). In contrast, all of the major "I-labeled proteins were concentrated to some degree by affinity chromatography on Con A (lane C), RCA (lane D), and WGA (lane F). Con A, however, bound less of the 135-kd protein (lane C); the run through from the Con A column contained significantly less of this protein (lane J). Negligible amounts of protein were present in the run through of the RCA and WGA columns (lanes K and M, respectively). Although the 185- and 115-kd proteins were slightly enriched by UEA chromatography (lane E), the run through (lane L) did not appear significantly different than that of a control agarose column (lane I).

**Immunoprecipitation of "I-labeled neutrophil proteins.** To further characterize the "I-labeled neutrophil surface proteins purified by lectin affinity chromatography, the proteins were immunoprecipitated with monoclonal antibodies OKM1, 60.3, and AHN-1 (Fig 2). Only eluates from the Con A, WGA, and RCA columns contained sufficient
Fig 1. Lectin affinity chromatography and polyacrylamide gel electrophoresis of $^{125}$I-labeled neutrophil cell-surface proteins. Neutrophils were labeled with $^{125}$I at the cell surface, solubilized, and fractionated by lectin affinity chromatography as described in the text. $^{125}$I-labeled proteins were analyzed by SDS-PAGE in a 10% gel and autoradiography as described in the text. Equal proportions of the material that passed through the columns and the eluates were applied to each lane. (Lane A) cell extract before affinity chromatography; (lanes B-H) proteins eluted from lectin affinity columns; (lanes I-O) proteins that passed through lectin affinity columns. (Lanes B and I) uncoupled agarose beads, (lanes C and J) Con A; (lanes D and K) RCA; (lanes E and L) UEA; (lanes F and M) WGA; (lanes G and N) PNA; (lanes H and O) DBA. Proteins used as molecular weight standards were myosin heavy chain, 200,000; Escherichia coli $\beta$-galactosidase, 116,000; phosphorylase B, 97,400; bovine serum albumin, 66,000; ovalbumin, 45,000; and carbonic anhydrase, 29,000.

Fig 2. Immunoprecipitation and polyacrylamide gel electrophoresis of $^{125}$I-labeled neutrophil surface proteins fractionated by lectin affinity chromatography. Neutrophils (1 to $2 \times 10^7$) were labeled at the cell surface with $^{125}$I, solubilized, and fractionated by lectin affinity chromatography (300 $\mu$L bed volume) and eluted with 400 $\mu$L of the appropriate buffer as described in the text. Equal volumes of eluate from each lectin column and cell extract were used for each immunoprecipitation reaction. (Lanes A-D) cell extract before affinity chromatography; (lanes E-P) proteins enriched by lectin chromatography with Con A (lanes E-H), WGA (lanes I-L) and RCA (lanes M-P). Immunoprecipitation was done using: OKM1 (lanes A, E, I, M), 60.3 (lanes B, F, J, N), AHN-1 (lanes C, G, K, O), and NMS (lanes D, H, L, P), and analyzed by SDS-PAGE in a 7.5% gel and autoradiography as described in the text. Molecular weight standards were the same as in Fig 1.
radioactivity to be analyzed by this technique. Equal volumes of eluate from each lectin column, column run through, and cell extract were used for each immunoprecipitation reaction. As expected, the CD11b antibody, OKM1, immunoprecipitated a 165-kd protein as well as a small amount of a 105-kd protein (seen better with longer exposures of the autoradiograph) from unfractionated cell extract (lane A). Antibody 60.3 (CD18) immunoprecipitated proteins of 185-, 165-, 142-, and 105-kd (lane B) corresponding to the αL, αM, αX, and β chains, respectively, of the LFA-1/HVMac-1/gp150,95 adhesion-promoting glycoprotein family. The CD15 antibody, AHN-1, immunoprecipitated major 125I-labeled proteins of 220-, 185-, 165-, 135-, and 105-kd (lane C). No radiolabeled proteins were immunoprecipitated with NMS (lane D).

When immunoprecipitation was performed using the Con A column eluate, faint but clearly defined 165-kd and 105-kd proteins were visible with OKM1 (lane E). These proteins were better visualized on longer exposures of the autoradiograph; the exposure time shown was chosen to allow clear visualization of the darker bands of the accompanying lanes and to allow comparison of the relative intensities of these bands. The 185-, 165-, 142-, and 105-kd proteins were seen more prominently following immunoprecipitation with 60.3 (lane F). Similarly, the 220-, 185-, 165-, 135-, and 105-kd proteins were also immunoprecipitated by AHN-1 (lane G), while no radiolabeled proteins were immunoprecipitated by NMS (lane H).

All proteins immunoprecipitated from the cell extract by these monoclonal antibodies could also be immunoprecipitated from the WGA and RCA column eluates (lanes I through P). The quantity of 125I-labeled proteins immunoprecipitated from the WGA and RCA eluates was significantly greater than that immunoprecipitated from the Con A eluate. In addition, a higher molecular weight band was sometimes seen with immunoprecipitation with AHN-1 in both the WGA (lane K) and RCA (lane O) eluates.

Immunoprecipitation was also done with the anti-CR1 monoclonal antibody, YZ-1 (Fig 3). As expected, YZ-1 (lane A), but not NMS (lane H), immunoprecipitated two 125I-labeled proteins from cell extract.34 These proteins were readily immunoprecipitated by YZ-1 from material that was enriched by Con A affinity chromatography (lane D), and to a lesser degree from the WGA eluate (lane B). No 125I-labeled protein was immunoprecipitated by YZ-1 from the RCA (lane C) or DBA (lane E) eluates. As expected, CR1 was immunoprecipitated by YZ-1 from the run through of the DBA column (lane G), but not from the Con A column run through (lane F).

125I-labeled alveolar macrophages. Alveolar macrophages obtained from bronchoalveolar lavage were also radiolabeled with 125I by the lactoperoxidase technique and fractionated by Con A, RCA, UEA, and WGA affinity chromatography. As previously described, three major 125I-labeled proteins of 183-, 80-, and 30-kd were reproducibly identified in the cell extract (Fig 4, lane A).23 The 183-kd protein has recently been identified as a mannose-receptor and the 30-kd protein has been found to be induced by cigarette smoking.23 The 183-, 80-, and 30-kd proteins were all enriched by Con A chromatography (lane B); little of these proteins was observed in the run through (lane F). The RCA (lane C) and WGA (lane E) column eluates also contained the 183- and 80-kd proteins, but the WGA column eluate contained little of the 30-kd protein. RCA column...
chromatography appeared to fractionate the 30-kd protein into an ~29-kd protein present in the RCA eluate (lane C) and a slightly higher molecular weight protein in the RCA column run through (lane G). No 125I-labeled proteins were detected in the UEA column eluate (lane D).

Whole cell extract. To further characterize the lectin-binding proteins of neutrophils and alveolar macrophages, unlabeled cell extracts were also analyzed by lectin affinity chromatography and SDS-PAGE, and the proteins were detected by Coomassie brilliant blue staining. The cell extract from normal neutrophils from a leukapheresis sample (Fig 5) contained greater than 40 cleanly distinguishable proteins (lane A). The protein compositions of the material that passed through the lectin columns (lanes F through I) were not strikingly different from the cell extract. The major protein seen in the eluate from the WGA column (lane B) was of ~98- to 105-kd. The UEA column eluate (lane C) contained several faintly staining proteins including three of ~120-, 105-, and 66-kd. Seven major proteins of ~200-, 185-, 98- to 105-, 90-, 55-, 53-, and 50-kd were seen in the RCA column eluate (lane D), and five major proteins of 180-, 120-, 105-, 98-, and 90-kd were enriched by Con A affinity chromatography (lane E). No proteins were enriched by chromatography on plain agarose (not shown). The lectin fractionation of these major proteins was readily reproducible. The lectin binding properties of these proteins did not clearly correlate with those of the major 125I-surface-labeled proteins seen in Fig 1.

The whole cell extract of alveolar macrophages (Fig 6) also contained multiple proteins (lane A). The eluate from the WGA (lane B) and RCA (lane D) columns contained a prominent 183-kd protein; the RCA column eluate also contained a distinct ~30-kd protein. While there was no clear difference between the proteins that passed through the WGA, UEA, and RCA columns (lanes F through H) and the unfractionated cell extract (lane A), the Con A column run through (lane I) was noticeably lacking the 30-kd protein. The lectin-binding patterns of the 183- and 30-kd proteins detected by Coomassie brilliant blue staining correlated well with the binding of 125I-surface-labeled proteins (Fig 4) except that the 183-kd protein was more prominent in the Con A eluate from the radiolabeled cell extract.

DISCUSSION

Plant lectins, especially Con A, are often used as stimuli of neutrophil and macrophage function and as probes of changes in cell membrane structure. Con A binding to the surface of neutrophils and macrophages results in a number of alterations of cell function and structure. Con A induces a respiratory burst with increased activity of the hexose monophosphate shunt, increased oxygen consumption, and the production of O2 and other oxygen-derived products, and induces capping of cell membrane molecules in neutrophils. Neutrophils that have been treated with Con A also selectively discharge specific granules. WGA and derivatives of WGA inhibit FMLP-induced neutrophil chemotaxis.

Fig 4. Lectin affinity chromatography and polyacrylamide gel electrophoresis of 125I-labeled alveolar macrophage surface proteins. Alveolar macrophages were surface labeled with 125I, solubilized, and fractionated by lectin affinity chromatography and analyzed by SDS-PAGE in a 10% gel and autoradiography as described in the text. Equal proportions of the material passed through the columns and eluates were applied to each lane. (Lane A) cell extract before affinity chromatography; (lanes B-E) proteins enriched by lectin chromatography; (lanes F-I) proteins present in column run through. Lectins were Con A (lanes B and F), RCA (lanes C and G), UEA (lanes D and H), and WGA (lanes E and I). Molecular weight standards were the same as in Fig 1.
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Fig 5. Lectin affinity chromatography and polyacrylamide gel electrophoresis of normal human neutrophils. Neutrophils were treated with DFP, solubilized, and fractionated by lectin affinity chromatography, analyzed by SDS-PAGE in a 5% to 15% gradient polyacrylamide gel, and visualized with Coomassie brilliant blue staining as described in the text. (Lane A) cell extract before affinity chromatography; (lanes B-E) proteins enriched by lectin chromatography; (lanes F-I) proteins that passed through lectin columns. (Lanes B and F) WGA; (lanes C and G) UEA; (lanes D and H) RCA; and (lanes E and I) Con A. Molecular weight standards were the same as in Fig 1.

the membrane potential and resistance, and inducing vacuole formation.

While the effects of these lectins on cell structure and function are presumably mediated by interaction of the lectins with cell surface molecules, the proteins involved have not been well characterized. The major Con A binding protein of rabbit neutrophils was shown to be a 140-kd protein as determined by overlaying SDS-PAGE gels of whole neutrophils with 125I-labeled Con A. Con A has also recently been shown to bind to the LFA-1/HMac-1/gp 150,95 adhesion glycoprotein family on human neutrophils. A derivative of WGA that inhibits reexpression of FMLP receptors on human neutrophils has been found to bind to a 62-kd membrane protein.

This study evaluated the binding of Con A, WGA, RCA, and UEA to both human neutrophils and alveolar macrophages as well as the binding of DBA and PNA to neutrophils. The carbohydrate specificity of each lectin is shown in Table 1. Con A was found to bind to members of the LFA-1/HMac-1/gp 150,95 adhesion glycoprotein family on human neutrophils; CD18 monoclonal antibodies immunoprecipitated the αL, αM, αX, and β chains of this protein family from an extract of neutrophils fractionated by Con A affinity chromatography. These results are in agreement with recent studies reporting the interaction of Con A with the HMac-1 (αM) and gp 150,95 (αX) members of this glycoprotein family from neutrophils. In addition, Con A was also found to bind to the C3b receptor, CR1, as identified by immunoprecipitation with YZ-1, as well as a 135-kd surface protein recognized by CD15 monoclonal antibodies. WGA and RCA also bound members of the LFA-1/HMac-1/gp 150,95 adhesion glycoprotein family and the 135-kd protein immunoprecipitated by CD15 antibodies, but had much less avidity for CR1.

Stimulus-specific desensitization of neutrophils is well described. The results reported here demonstrating that Con A binds to CR1 and to members of the LFA-1/HMac-1/gp 150,95 adhesion glycoprotein family, including the αM chain that has been strongly implicated in CR3 function, coupled with previous studies demonstrating stimulus-specific desensitization, may partially explain the observation that neutrophils previously stimulated by
Fig 6. Lectin affinity chromatography and polyacrylamide gel electrophoresis of human alveolar macrophages. Alveolar macrophages obtained by bronchoalveolar lavage were treated with DFP, solubilized, fractionated by lectin affinity chromatography, analyzed by SDS-PAGE in a 5% to 15% gradient polyacrylamide gel, and visualized with Coomassie brilliant blue staining as described in the text. (Lane A) cell extract before affinity chromatography; (lanes B-E) proteins eluted from lectin columns; and (lanes F-I) proteins that passed through lectin columns; (lanes B and F) WGA; (lanes C and G) UEA; (lanes D and H) RCA; and (lanes E and I) Con A. Molecular weight standards were the same as in Fig 1.

Con A no longer respond to opsonized zymosan but are responsive to FMLP and the tumor promoter phorbol myristate acetate. Since Con A binds CR1 and CR3, appropriate stimulus by Con A might be expected to desensitize the cell to further stimulation by agents that act through these same receptors, but allow response to agents that act through different receptors.

The three major surface-labeled proteins of alveolar macrophages all bound Con A, including the 183-kd mannos receptor and the 30-kd smoking-associated protein. RCA and WGA also bound the 183-kd protein. WGA did not bind the 30-kd protein, while RCA appeared to fractionate the 30-kd protein into an ~29-kd protein that bound to RCA and an ~30-kd protein that did not.

These studies demonstrate that Con A and WGA interact with a number of functionally important cell-surface glycoproteins on human neutrophils and alveolar macrophages. These lectins may also react with functionally important cell-surface glycoproteins not identified by this radiolabeling technique. Although not tested in this study, these lectins likely interact with cell-surface glycolipids as well, an interaction that may also affect cell function and structure. Further isolation and analysis of the proteins involved in lectin binding should aid in the interpretation of studies on the effects of lectins on neutrophils, macrophages, and other cells. The lectin fractionation techniques described here may be of further use in devising purification schemes for these proteins.

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