Isolation and Characterization of β-Glucan Receptors on Human Mononuclear Phagocytes

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Summary

β-glucan receptors, with ligand specificity for yeast and fungal carbohydrate polymers, have been studied as phagocytic receptors of human monocytes. To characterize their structure, binding studies were carried out with human U937 cells and a rabbit IgG anti-Id that recognizes epitopes on monocyte β-glucan receptors. Unstimulated U937 cells specifically bound large amounts of the anti-Id, but almost none of the control anti-isotype. At saturation, the number of anti-Id molecules bound per U937 cell was 2.6×10^6 with an apparent K_a of 1.9×10^7 M^{-1}. Immunoprecipitates from detergent lysates of surface-radioiodinated U937 cells contained only two membrane proteins with antigenic specificity for the anti-Id, one having a mol wt of 180 kD and the other 160 kD. Both proteins were disulfide-linked and presented, after reduction, as five polypeptides of 95, 88, 60, 27, and 20 kD. Detergent lysates of unlabeled U937 cells, purified by affinity chromatography on anti-Id-Sepharose, yielded the same two nonreduced proteins and five reduction products in slab gels stained with Coomassie blue. In Western blots probed with the anti-Id, the most immunoreactive nonreduced and reduced affinity-purified products were the 160 and 20 kD molecules, respectively. Immunoblots of two-dimensional gels showed the 180 and 160 kD proteins to express a common epitope through disulfide linkage to the 20 kD polypeptide. By immunoblot analysis, U937 cell glucan-binding proteins from detergent lysates contained two cell proteins antigenic for the anti-Id that were indistinguishable from affinity-purified molecules in size and subunit composition. Studies of affinity-purified proteins from detergent lysed human monocytes were characterized by immunoblot analysis and found to be identical to U937 cell β-glucan receptors. They consisted of two disulfide-linked proteins, with mol wt of 180 and 160 kD, and had in common a 20 kD polypeptide with the anti-Id epitope.

β-glucan receptors (1) were first identified on human monocytes as phagocytic receptors which initiate phagocytosis of particulate activators of the human alternative complement pathway in the absence of opsonins (2). Subsequent studies principally with zymosan and glucan particles, have shown that human alveolar macrophages (3), neutrophils (4–6), eosinophils (7), and murine macrophages (8, 9) possess phagocytic receptors of comparable ligand specificity for the β-glucans commonly present in yeasts and fungi (10). Pathogens such as candida and aspergilli contain "yeast" glucan (11), cell wall components consisting of branched homopolymers of β-D-glucose with 1,3-consecutive and 1,6-crosslinked chains (12) and prototypic of *Saccharomyces cerevisiae* (10). Particulate yeast glucan is similar in size and glucose composition to zymosan particles (13, 14), but different in carbohydrate and protein content, with glucan particles being a purer form of zymosan (12, 15).

The smallest functional unit ligand for human monocyte β-glucan receptors has been isolated from purified yeast glucan and shown by mass spectrometry to be a heptaglucoside (16). The recent development of rabbit anti-idiotypic antibodies to an Id of a mAb with specificity for the yeast heptaglucoside has provided the first immunologic probe that recognizes epitopes on monocyte β-glucan receptors (17). The anti-Id specifically binds to human monocytes and selectively blocks their ingestion of zymosan and glucan particles. In the current studies, we determine the relationship between U937 cell proteins with the anti-Id epitope and β-glucan receptors by comparing the proteins eluted from anti-Id-Sepharose to those from yeast glucan particles. Both reagents identify the

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same two species of molecules in U937 cells and these are also present in human monocytes.

Materials and Methods

Chemicals and Reagents. Diisopropyl fluorophosphate (DFP),[1] NP-40, PMSF, pepstatin, leupeptin, and general chemicals were obtained from Sigma Chemical Co. (St. Louis, MO); acrylamide, SDS, glycine, nitrocellulose, and other electrophoretic supplies from Bio-Rad Laboratories (Richmond, CA); and the human myelomonocytic U937 cell line and murine OKM1 hybridoma from the American Type Culture Collection (Rockville, MD).

Cell Culture and Isolation. U937 cells were cultured in 150 cm^2 tissue culture flasks (Costar Corp., Cambridge, MA) containing RPMI 1640 Medium (Gibco Laboratories, Grand Island, NY) and 10% heat-inactivated (56°C for 30 min) calf serum (Gibco Laboratories). The cell cultures were incubated at 37°C in a humidified atmosphere of 5% CO_2 and harvested during logarithmic phase of growth by centrifugation. As specified in the text, the cells were washed 3–5 times in HBSS, which lacked calcium, magnesium, and phenol red, or in RPMI. They were resuspended in buffer or medium, counted on a Coulter counter (Coulter Electronics, Hialeah, FL), and measured for viability by Trypan blue exclusion, which was ≥95%.

Human monocytes were isolated (2) from normal citrated and dextran-treated blood, purified by gradient centrifugation on Ficoll-Paque (Pharmacia Fine Chemicals, Piscataway, NJ), washed in HBSS, and resuspended in RPMI containing 1 mg/ml BSA (Miles Laboratories, Elkhart, IN). Monolayers of monocytes were prepared (17) in 60-mm plastic tissue culture dishes (Becton Dickinson and Co., Oxnard, CA); 1.5 ml of 2.2 × 10^6/ml mononuclear cells were used in each of two layerings. By visual enumeration at ×40 with an inverted phase contrast microscope and a calibrated reticle, 20–35% of the layered mononuclear cells adhered to the dishes. By morphology and nonspecific esterase staining, ≥95% of the adherent cells were monocytes.

Antibodies. Mouse IgG2a mAb UPC 10 with specificity for β-fructans (Organon Teknika Corp., Durham, NC), nonimmune rabbit IgG (Miles Inc., West Haven, CT), and goat IgG anti-rabbit $F(ab')_2$ (Cappel Laboratories, Westchester, PA) were purchased; rabbit IgG anti-CR1 (18) was kindly provided by Dr. Richard Jack (Harvard Medical School, Boston, MA).

Mouse hybridomas producing IgG2a mAb OEA10 and IgG1 OKM1 with specificities for yeast β-glucans and the α chain of CD11b, respectively, were raised in spinner cultures; the mAb were purified by affinity chromatography with rat mAb AHF5 anti-mouse L chain (17). The anti-Id, as described in detail (17), was raised in rabbits immunized with mAb OEA10 and rendered specific for Id by adsorption of mouse serotypic and isotypic determinants before affinity purification by passage and elution from Sepharose-mAb OEA10 with 0.1 M glycine HCl, pH 2.5. Rabbit anti-mouse IgG2a was eluted from Sepharose-mAb UPC 10. By SDS-PAGE, the purified anti-Id and corresponding anti-isotype contained only IgG.

Radioiodination of Antibodies. Antibody labeling was carried out by the chloroglycouril method (19) with 100-200 μg of IgG protein, 1-2 mCi of carrier-free $Na^{125}I$ (New England Nuclear, Boston, MA), and IODO-BEADS (Pierce Chemical Co., Rockford, IL). Iodinated antibody was separated from free iodide by gel-filtration through Sephadex G-25 (Pharmacia Fine Chemicals) columns in PBS and 0.02% NaN_3. The specific activity of the radiolabeled antibodies was 1–2 × 10^6 cpm/μg.

Protein-Coupled Sepharose Beads and Purified Glucan Particles. BSA, OKM1, nonimmune rabbit IgG, anti-isotype, and anti-Id were each coupled in 0.1 M phosphate buffer, pH 7.0, at a concentration of 4 mg of protein/g of activated CH-Sepharose beads (Pharmacia Fine Chemicals) with coupling efficiencies of 75–85%. For anti-CR1, the proportion of protein to beads was reduced by half and 82% of the protein was covalently bound to the beads.

The preparation of purified glucan particles from *S. cerevisiae* (Fleischmann, E. Hanover, NJ) was the same as that used in previous studies (17). The yeast were treated sequentially with hot NaOH, hot acetic acid, ethanol, and acetone, and the final product dried under vacuum. After resuspension, the particles were counted and analyzed for carbohydrate and protein (16). 1 mg of glucan contained 10^8 particles, 99% carbohydrate, 0.6% protein, and no neutral sugar other than glucose.

Binding Studies with U937 Cells. Suspensions of 4 × 10^5 U937 cells, which had been washed in RPMI, were incubated in 0.30 ml of cold RPMI containing 10 mM Hepes, 300 μg of BSA, 40 μg of nonimmune rabbit IgG, and increasing amounts of ^{125}I-rabbit IgG anti-Id for 90 min at 4°C, which was sufficient to reach equilibrium. Nonspecific binding was assessed by incubating samples in the presence of 40- to 400-fold molar excess of unlabeled anti-Id. Replicate samples of cells in 0.075 ml were layered on 0.25 ml of a 3:1 mixture of dibutyl/dinonyl-phalate (ICN Biomedicals Inc., Plainview, NY) in 0.5 ml polypropylene microfuge tubes and centrifuged at 8,000 *g* for 1 min. The tubes were cut, and the pellets and supernatants measured for cell-bound and free ^{125}I-anti-Id, respectively. The specific binding data were analyzed by the LIGAND computer program (20) to determine the affinity and number of bound molecules per cell at saturation. Binding of ^{125}I-rabbit IgG anti-mouse IgG2a (UPC 10) was carried out in a smaller manner in the absence and presence of 40-fold excess unlabeled anti-isotype.

Radioiodination and Immunoprecipitation of Surface U937 Cell Proteins. U937 cells, washed five times in HBSS, were surface-labeled for 1 h at 4°C by the incubation of 2.5 × 10^7 cells in 1 ml of HBSS and 1 mCi of $Na^{125}I$ (New England Nuclear) in glass vials coated with 150 μg of IODO-GEN (Pierce Chemical Co.). The labeled cells were centrifuged at 700 *g* for 4 min at 4°C, washed three times in cold HBSS containing 2 mg/ml BSA and twice in buffer alone, and then lysed for 1 h at 4°C in 8 ml of HBSS containing 1% NP-40, 5 mM DFP, 2 mM PMSF, 1 μM pepstatin, and 1 μM leupeptin (lysis buffer). The lysates were centrifuged at 10,000 *g* for 1 h at 4°C and the resulting supernatant fractions assessed for radiolabeled protein. Of the original radioiodide, 6.2 ± 1.7% (mean ± SD, n = 7) was incorporated into cells and 2.7 ± 1.0% was precipitable by TCA.

For immunoprecipitation, the detergent-soluble materials were incubated for 18 h at 4°C with Sepharose-BSA and the precleared lysates sequentially incubated for 1 h at 4°C with 100 μl of the packed protein-coupled Sepharose beads indicated in each study. The beads were washed five times in lysis buffer, treated with 300 μl of 1% SDS for 5 min at 100°C to elute adsorbed proteins, and sedimented by centrifugation at 700 *g* for 5 min at 25°C. Eluted soluble materials were centrifuged at 14,000 *g* for 5 min at 10°C, lyophilized, dissolved in Laemmli sample buffer (21), and subjected to SDS-PAGE as described below. Radioautographs were prepared by exposing dried gels to X-ray film (XAR X-Omat; Eastman Kodak Co., Rochester, NY).

Unlabeled Cell Lysates. Batches of 6.4 ± 2.0 × 10^8 U937 cells (mean ± SD, n = 21) were harvested and washed four times in

[1] *Abbreviations used in this paper:* DFP, diisopropyl fluorophosphate; DTT, dithiothreitol.

HBSS. Pelleted cells were resuspended at a density of 5×10^6 cells/ml of lysis buffer, incubated for 1 h at 4°C with frequent agitation, and stored at −70°C. Immediately before use, the lysates were centrifuged at 10,000 *g* for 1 h at 4°C to remove detergent-insoluble materials.

For monocyte lysates, replicate dishes of buffer-washed adherent cells were each treated with 1.5 ml of lysis buffer and scraped with a disposable cell scraper (Costar Corp.). Examination of the dishes by inverted phase microscopy revealed nuclei but few intact cells. To maximize protein yield, sets of dishes (29–50) with individual donor monocytes were treated with lysis buffer already containing solubilized cells. The final pooled product was incubated for 1 h at 4°C, stored at −70°C, and clarified by centrifugation before use.

Immunoadsorption and Glucan-Binding. For immunoadsorption, detergent-soluble materials of batch-lysed U937 cells and monocytes were precleared as before with Sepharose-BSA and the precleared products sequentially incubated with Sepharose beads bearing nonimmune rabbit IgG and anti-Id. The beads were washed and eluted, as described for radiolabeled immunoprecipitates, and the final soluble products were stored at −70°C as lyophilized powders.

For glucan-bound materials, replicate samples of detergent-soluble lysates of 6.5×10^7 U937 cells were precleared with Sepharose-BSA and incubated for 4 h at 4°C with 6.5×10^8 glucan particles. To obtain adequate amounts of protein, washed particles from 3–4 samples were pooled before elution and subsequent lyophilization. For studies in which β-glucan receptors and proteins with the anti-Id epitope were directly compared, parallel samples of immunoadsorbed proteins were prepared in a similar manner.

Immunoaffinity Column Chromatography. For affinity purification of U937 cell proteins, 750–900 ml of detergent-soluble fractions from $3–4 \times 10^9$ lysed cells and with 0.02% NaN_3 were chromatographed sequentially on columns of Sepharose 4B (6 × 2.5 cm), nonimmune rabbit IgG-Sepharose (4.5 × 2.5 cm), and anti-Id-Sepharose (4.0 × 2.5 cm) at a flow rate of 20 ml/h at 10°C. Proteins were continuously monitored by OD at 280 nm with an on-line UV-detector (Isco, Lincoln, NE). The columns were washed in 500–750 ml of PBS with 0.02% azide at a rate of 35 ml/h. To remove azide and to establish baselines, the anti-Id-Sepharose was washed in 100–150 ml of PBS before elution of bound materials with 0.1 M glycine-HCl, pH 2.5. The manually collected proteins were dialyzed at 4°C against 1 mM PO_4, 7.5 mM NaCl, pH 7.0, lyophilized, dissolved in distilled water, and stored at −70°C.

Purification of monocyte proteins were carried out in a similar fashion with 60–90 ml of detergent-soluble fractions from $4–7 \times 10^8$ lysed monocytes.

SDS-PAGE. SDS-PAGE was performed as described (22) in 1.5-mm discontinuous slab gels, a 3% gel stacked on a 5–15% polyacrylamide gradient resolving gel. Protein samples were prepared by heating at 100°C for 5 min in Laemmli sample buffer containing 1% SDS with or without 0.1 M dithiothreitol (DTT). To establish the apparent mol wt of cell proteins, prestained standards (Bethesda Research Laboratories, Gaithersburg, MD) were used as marker proteins. The prestained standards consisted of myosin (200 kD), phosphorylase b (97 kD), BSA (67 kD), OVA (43 kD), carbonic anhydrase (29 kD), β-lactoglobulin (18 kD), and lysozyme (14 kD).

Two-Dimensional Electrophoresis. For nonreducing/reducing two-dimensional SDS-PAGE, immunoaffinity purified proteins were heated in sample buffer with 1% SDS, loaded into 5-mm wells of gels, and electrophoresed. Gel strips, 11 × 2 cm, containing the resolved proteins were excised, incubated at 25°C for 1 h in sample buffer with 1% SDS and 0.1 M DTT, and inserted into a 13-cm sample well of the second gel; prestained standards were loaded into a separate 7-mm well. The running buffer for the second gels contained 0.1 mM sodium thioglycollate.

Immunoblotting. Proteins resolved by SDS-PAGE were transferred onto nitrocellulose, analyzed by the immunoblotting method described (22) with 25 μg/ml of anti-Id and 10^5 cpm/ml of ^{125}I-goat anti-rabbit $F(ab')_2$, and detected by radioautography on X-ray film. The primary and secondary antibodies were diluted in 0.01 M Tris, 0.15 M NaCl, 0.02% NaN_3, pH 7.4, containing 2% BSA (Tris-BSA). In the absence of anti-Id, blots incubated in Tris-BSA with or without 25 μg/ml of nonimmune rabbit IgG contained no detectable proteins.

Radioautographic Method for Protein Determination. To conserve isolated cell protein, serial dilutions of affinity-purified proteins were spotted in 2.5 μl onto nitrocellulose, detected by direct probing with ^{125}I-anti-Id in radioautographs, and quantitated by densitometry with mAb OEA10, the immunogen for the anti-Id, as reference standard. To assess for purity, diluted samples on replicate strips were treated in a similar fashion with labeled nonimmune rabbit IgG or goat anti-rabbit $F(ab')_2$ and the concentrations of detected protein calibrated against unlabeled anti-Id. The purity of isolated cell proteins with the anti-Id epitope was ≥95%. Control blots probed with ^{125}I-nonimmune IgG showed no cell or reference protein. For three separate preparations, the yields of affinity-purified U937 cell protein were 1.8 ± 1.0 μg (mean ± SD) per 10^8 lysed cells. Similar concentrations of cell protein were obtained by indirect probing with unlabeled anti-Id and detection with the labeled goat antibody.

Results

Binding of the Anti-Id to U937 Cells. The anti-Id is specific for the Id of mAb OEA10 anti-yeast β-glucans and cross-reactive with epitopes found on human monocyte β-glucan receptors (17). To determine whether comparable epitopes were expressed by U937 cells, preliminary binding studies were carried out with duplicate sample mixtures containing increasing doses of ^{125}I-anti-Id or ^{125}I-anti-isotype in the absence and presence of 40-fold excess of the corresponding unlabeled antibody. U937 cells exhibited substantial amounts of specific binding of the anti-Id, which approached plateau levels of 8% at an input of 1 μg of labeled antibody, and low levels of binding of the anti-mouse isotype, which remained constant irrespective of dose (data not shown).

Binding of the anti-Id to U937 cells was further evaluated with duplicate sample mixtures containing 1 μg of ^{125}I-anti-Id and increasing amounts of unlabeled anti-Id or nonimmune IgG, which ranged from 0 to 400 μg. The average percentage of bound ^{125}I-anti-Id, initially 7.76%, was progressively decreased by the unlabeled antibody and unaffected by nonimmune IgG (Fig. 1). In the presence of 100 and 200 μg of unlabeled anti-Id, binding by U937 cells was reduced to averages of 0.64 and 0.53%, respectively. Specific binding was determined by subtracting the percentage of cell-bound radioactivity in the presence of unlabeled anti-Id at a 400-fold molar excess, multiplying the resulting proportions by the total antibody added, and analyzing the data in Scatchard plots. U937 cells bound 0.66 μg of anti-Id per 10^6 cells, equivalent to approximately 2.6×10^6 IgG molecules per cell, and these had an apparent K_a of 1.9×10^7 M^{-1} (Fig.

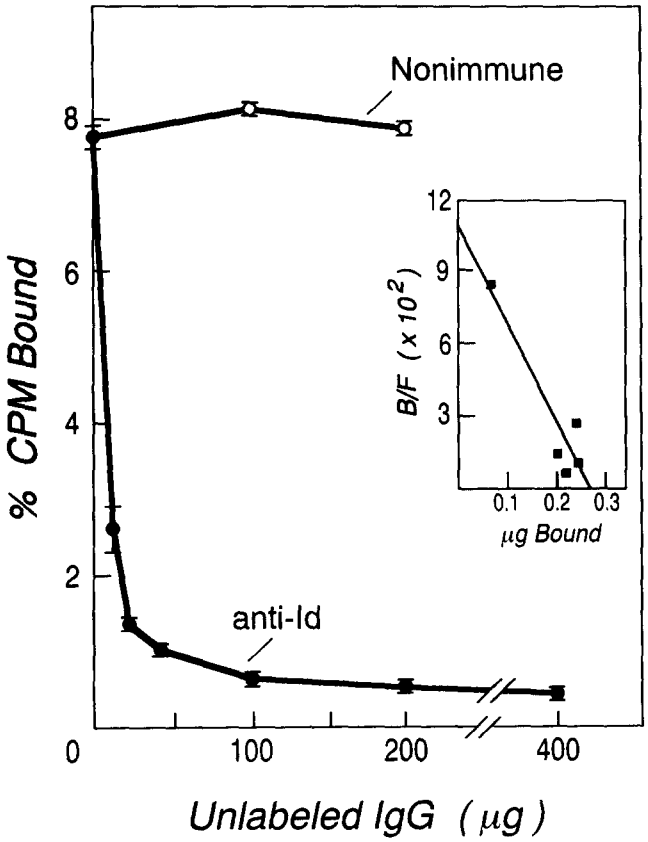

Figure 1. Equilibrium binding of rabbit IgG anti-Id to unstimulated U937 cells. Suspensions of 4 × 10^5 cells were incubated for 90 min at 4°C with 1 μg of ^{125}I-anti-Id in the presence of increasing amounts of unlabeled anti-Id (●) and nonimmune rabbit IgG (○). Cell-bound and free radiolabel were separated by centrifugation on oil. The data are plotted as the mean with range percent cpm bound, are duplicate determinations of two cell reactions, and are representative of three complete studies. The data point obtained with 400 μg of unlabeled anti-Id was used in calculations of specific binding (■) for Scatchard analysis (inset).

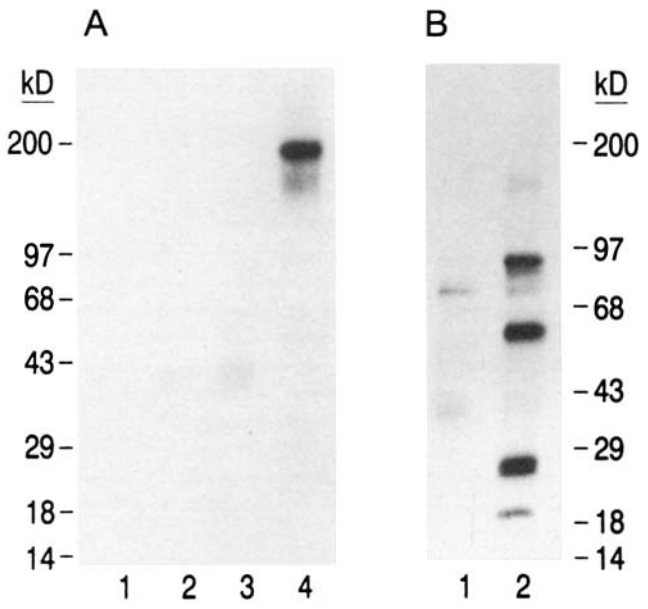

Figure 2. Surface expression of proteins with the anti-Id epitope by unstimulated U937 cells. Detergent-soluble proteins from surface-radioiodinated U937 cells were sequentially immunoprecipitated for 1 h each at 4°C with nonimmune rabbit IgG (lane *1*), OKM1 (lane *2*), anti-CR1 (lane *3*), and anti-Id (lane *4*). The bound materials were eluted and resolved in nonreduced samples on 5–15% acrylamide gradient slab gels by SDS-PAGE with run-off of the dye front (*A*). Surface proteins in reduced samples of eluates sequentially obtained from nonimmune IgG- (lane *1*) and anti-Id-Sepharose (lane *2*) were resolved in a similar manner (*B*). Dried gels of the nonreduced and reduced samples were exposed to X-ray film for 4 and 16 h, respectively. Mobility and size (kD) of prestained protein standards are indicated.

1, inset). Although the number of apparent binding sites was high, there were no significant differences in the number of surface antigenic sites or their affinity for the anti-Id with increased washing of harvested cells, preincubation of washed cells in HBSS for 2–4 h, or use of ultracentrifuged preparations of anti-Id. These data indicated U937 cells to be a rich source of proteins with the anti-Id epitope and possible specificity for yeast β-glucans.

Immunoprecipitation of Surface-Labeled U937 Cell Proteins. Biochemical studies of proteins with the anti-Id epitope were carried out with radioiodinated intact U937 cells, which were subsequently lysed. Detergent-soluble proteins, sequentially immunoprecipitated by nonimmune rabbit IgG, OKM1, anti-CR1, and anti-Id, were resolved by SDS-PAGE and detected by radioautography. Two membrane proteins were specifically immunoprecipitated by the anti-Id: a prominent species of 180 kD and a slightly less intense molecule of 160 kD (Fig. 2 *A*). The detection of little or no protein antigenic for OKM1 or anti-CR1 was in agreement with other studies of these receptors on U937 cells (23, 24). Immunoprecipitations performed without preadsorptions by OKM1 and/or anti-CR1 showed the anti-Id epitope to be restricted to the same two proteins and provided further evidence for the high specificity of the anti-Id.

After reduction, the two membrane proteins with the anti-Id epitope showed several faintly detectable radioactive bands but little or no parent molecule. To demonstrate these more clearly, detergent-soluble proteins were prepared from four times as many surface-labeled cells and subjected, as a single batch, to sequential immunoprecipitation with nonimmune IgG- and anti-Id-Sepharose beads. Five prominent reduction products of the two immunospecific membrane proteins were detected: a 95 kD, an 88 kD, a 60 kD, a 27 kD, and a 20 kD (Fig. 2 B). None of these was detected in eluates from the nonimmune IgG-coupled beads.

Immunoaffinity Purification of Detergent-Soluble U937 Cell Proteins. To determine whether additional cellular proteins contained the anti-Id epitope, soluble proteins from 3–4 × 10^9 detergent-lysed cells were passaged through columns of nonimmune IgG-Sepharose followed by passage and elution from columns of anti-Id-Sepharose. The affinity-purified proteins were resolved in nonreduced and reduced samples by SDS-PAGE and detected by staining with Coomassie blue. Electrophoretic separation of an estimated 5 μg of purified protein yielded two major molecules of 180 and 160 kD, and

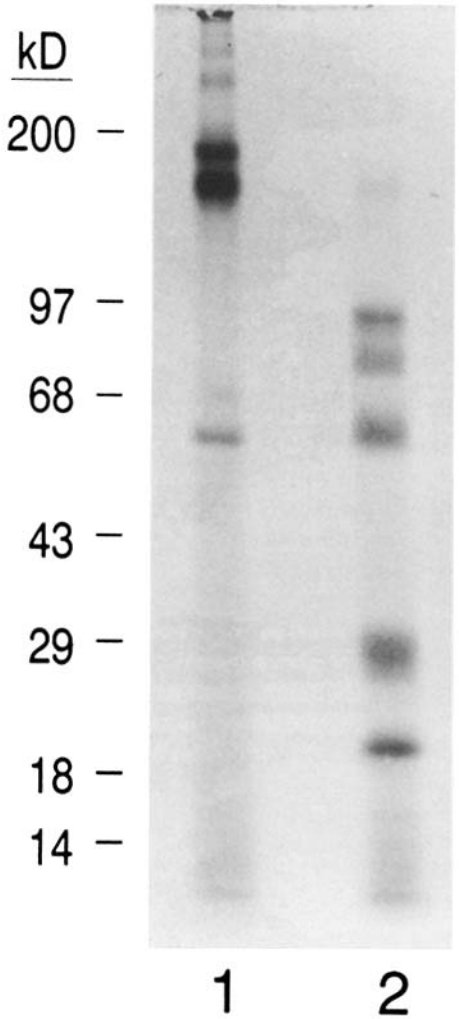

Figure 3. Expression of the anti-Id epitope by soluble U937 cell proteins. Proteins from detergent lysed U937 cells were purified by affinity chromatography on anti-Id-Sepharose, resolved without (lane *1*) and with (lane *2*) reduction in 5–15% acrylamide gradient slab gels by SDS-PAGE, and stained with Coomassie blue. Similar analysis of three preparations of purified cell proteins showed no additional molecules. Mobility and size (kD) of prestained standards are indicated.

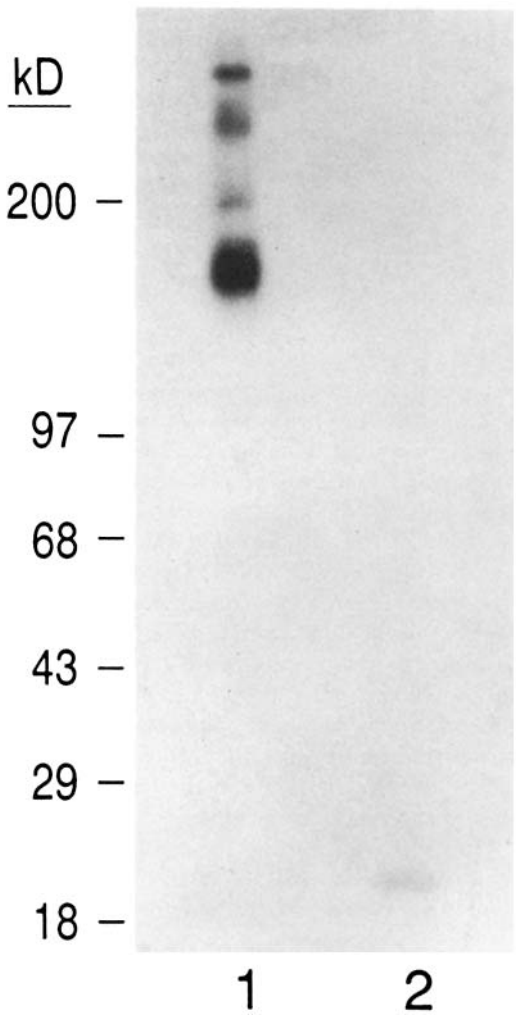

Figure 4. Immunoblot of affinity-purified U937 cell proteins with the anti-Id epitope. Nonreduced (lane *1*) and reduced (lane *2*) samples each with 0.4 μg of purified U937 cell proteins were subjected to SDS-PAGE, transferred onto nitrocellulose, probed with the anti-Id, and detected by radioautography with ^{125}I-goat anti-rabbit F(ab')$_2$; the film was exposed for 6 d. In duplicate blots, none of the proteins was reactive with nonimmune rabbit IgG or the labeled goat antibody. Mobility and size (kD) of prestained standards are indicated.

five prominent reduction products of 95, 88, 60, 27, and 20 kD (Fig. 3). By densitometry, the concentration of the 180 kD protein was approximately two-thirds that of the 160 kD, whereas the concentrations of the five reduction products were nearly equal. In addition to these proteins, nonreduced samples contained two apparent aggregates of high mol wt and a protein of 60 kD, which, as a group, accounted for about 15% of the total stained protein; reduced samples had a 5–7% content of a 160 kD stained protein. Analyses of three preparations of similarly purified proteins showed slightly different proportions of these minor constituents, but no additional molecules with the anti-Id epitope.

Immunoblot Analysis of Affinity-Purified U937 Cell Proteins. For resolution of the 180 and 160 kD proteins and comparison of their reduction products, nonreduced and reduced samples containing 0.4 μg of affinity-purified cell protein were subjected to SDS-PAGE, electrophoretically transferred onto nitrocellulose, and probed with the anti-Id. Under these conditions, the 180 and 160 kD proteins were clearly reactive with the anti-Id, but the only detectable protein after reduction was the 20 kD polypeptide (Fig. 4). Neither these molecules nor the apparent aggregates showed reactivity with nonimmune IgG or ^{125}I-goat anti-rabbit F(ab')$_2$ (data not shown).

Reactivity of the anti-Id with the 160 kD protein was always greater than that of the 180 kD. This was further confirmed by immunoblot analysis of two-dimensional gels. For this analysis, 4.8 μg of nonreduced affinity-purified protein were resolved in the first gel. These proteins were reduced in the second dimension, transferred onto nitrocellulose, and probed with the anti-Id in immunoblots. The anti-Id detected the

20 kD polypeptide, as found previously, and further demonstrated that this subunit component was a constituent of each of the nonreduced proteins, including all of the aggregated proteins (Fig. 5). Smaller amounts of reduced polypeptides of 95, 60, and 27 kD were also detectable, indicating that the anti-Id epitope was not limited to the 20 kD polypeptide.

Identification and Characterization of U937 Cell β-Glucan Receptors. To determine whether U937 cells had β-glucan receptors reactive with the anti-Id, samples of glucan particles were incubated with detergent-soluble proteins at a particle-to-cell ratio of 10:1, washed, and the eluted materials were analyzed in immunoblots. For comparison, soluble proteins from the same batches and numbers of lysed cells were immunoadsorbed with an equal ratio of packed anti-Id-Sepharose beads; nonreduced and reduced samples each containing half of the eluted proteins were analyzed concurrently with the glucan-derived materials. Under these conditions, the proteins eluted from anti-Id-Sepharose beads were markedly overloaded in nonreduced samples; however, all were clearly resolved by reduction and demonstrated to contain abundant quantities of the 20 kD polypeptide (Fig. 6, lanes *1* and *3*). Two distinct glucan-binding proteins were identified with the anti-Id, a minor protein of 180 kD and a major molecule of 160 kD; one prominent polypeptide of 20 kD was detected after reduction of equal amounts of protein (Fig. 6, lanes *2* and *4*). Control eluates from the same numbers of pooled buffer-treated glucan particles (2×10^9) contained no detectable protein in immunoblots probed directly or indirectly with either nonimmune IgG or anti-Id (data not shown). Despite the presence of large amounts of protein, none of the proteins bearing the anti-Id epitope from antibody- or glucan-derived samples was detected in duplicate blots probed with ^{125}I-goat anti-rabbit IgG with or without nonimmune IgG.

Identification and Characterization of Monocyte β-Glucan Receptors. To determine the molecular nature of human monocyte β-glucan receptors reactive with the anti-Id, detergent-soluble proteins in 3–7 × 10^7 adherent cells from individual monocyte donors were purified by adsorption of cell proteins to nonimmune rabbit IgG-Sepharose beads before passage and elution from anti-Id-Sepharose. The eluted proteins from both types of beads were resolved by SDS-PAGE and analyzed by immunoblotting with the anti-Id. Two monocyte proteins with mol wt of 180 and 160 kD and apparent aggregates of these proteins bound the anti-Id (Fig. 7 *A*, lane *2*). None of these species had specificity for nonimmune rabbit IgG (Fig. 7 *A*, lane *1*). Monocytes prepared from four separate donors always demonstrated a dominant band of 160 kD and, in one case, this was the only detectable monocyte protein reactive with the anti-Id. For quantitative comparison, the experiments designed to demonstrate the presence and structural properties of U937 cell β-glucan receptors (Fig. 6) were repeated, but the amounts of immunodetectable 160 kD protein in the antibody- and glucan-derived samples were normalized to each other and to the corresponding monocyte product. Electrophoretic separation of 0.1 μg and 3.0 μg of cell protein in antibody- and glucan-derived samples, respectively, yielded amounts of immunodetectable 160 kD proteins, which were similar (Fig. 7 *B*) and comparable to the depicted monocyte product (Fig. 7 *A*, lane *2*). In terms of

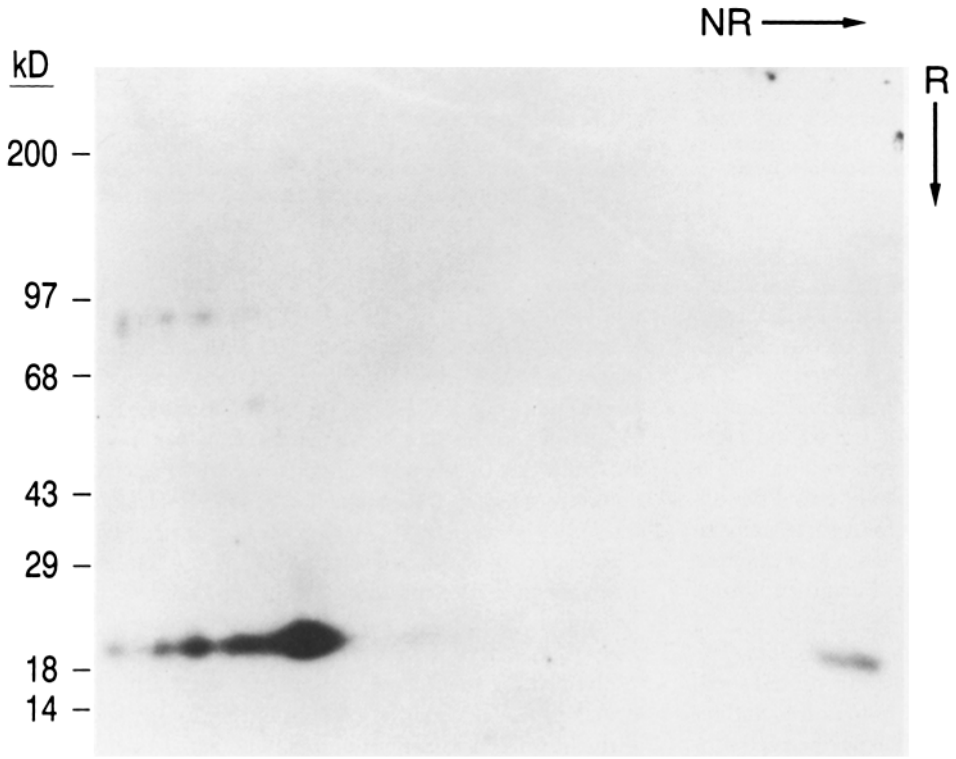

Figure 5. Subunit localization of the anti-Id epitope in purified U937 cell proteins. Two-dimensional SDS-PAGE was performed with 4.8 μg of affinity-purified U937 cell protein under nonreducing (NR) conditions in the first dimension and reducing (R) conditions in the second. Proteins were immunoblotted with the anti-Id and detected by radioautoradiography, as described for Fig. 4, after 17 h of exposure. Immunoblot analysis of three two-dimensional gels showed the same polypeptides with the anti-Id epitope; control blots revealed no molecules with specificity for nonimmune rabbit IgG or the labeled goat antibody.

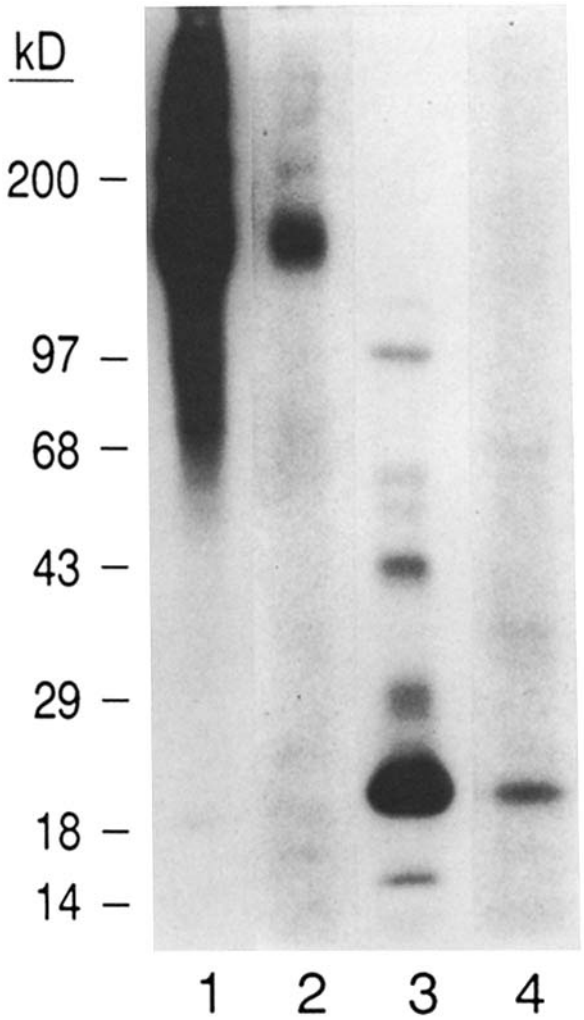

Figure 6. Identification and structure of U937 cell β-glucan receptors. Glucan-binding proteins from 2 × 10^8 detergent lysed U937 cells were evenly divided in nonreduced (lane *2*) and reduced (lane *4*) samples, resolved by SDS-PAGE, and analyzed in immunoblots probed with the anti-Id as described in Fig. 4; the film was exposed for 6 d. For comparison, soluble proteins from the same number of detergent-lysed cells were concurrently immunoabsorbed with an equal ratio of packed anti-Id-Sepharose beads and analyzed in immunoblots of nonreduced (lane *1*) and reduced (lane *3*) samples; the film was exposed for 17 h. The glucan- and antibody-derived materials were run in the same slab gel and the results are representative of five analyses.

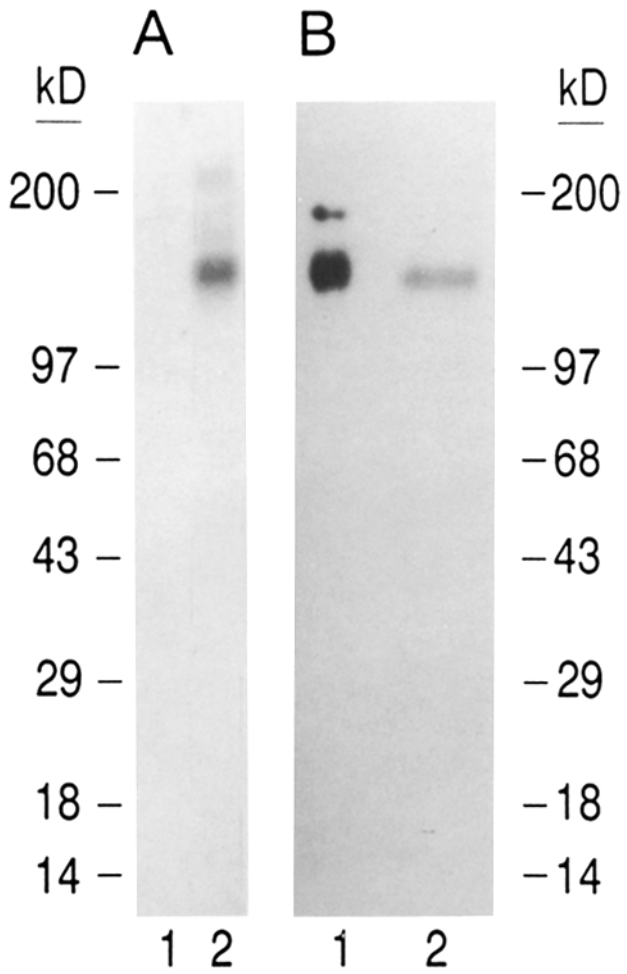

Figure 7. Comparison of β-glucan receptors on monocytes and U937 cells. Soluble detergent lysates of 6 × 10^7 adherent human monocytes were sequentially immunoprecipitated for 1 h at 4°C with Sepharose beads bearing nonimmune rabbit IgG (lane *1*) and anti-Id (lane *2*) and nonreduced samples of the eluated proteins were analyzed, as described in Fig. 4, in immunoblots probed with the anti-Id (*A*). For comparison, 0.1 and 3.0 μg of U937 cell protein eluated from anti-Id-Sepharose beads (lane *1*) and glucan particles (lane *2*), respectively, were similarly resolved by SDS-PAGE and immunoblotted with the anti-Id (*B*). Both films were exposed for 9 h; the data are representative of four individual monocyte donors.

cell number, these data suggested that monocytes contained 20–40 times fewer β-glucan receptors than U937 cells.

To determine whether the structural properties of β-glucan receptors in monocytes and U937 cells were similar, detergent-soluble proteins from 4–7 × 10^8 monocytes were immunopurified by column chromatography and compared, in reduced samples, to column-purified U937 cell proteins. Duplicate samples, each containing about 0.4 μg of monocyte and 0.8 μg of U937 cell protein, were subjected to SDS-PAGE and immunoblot analysis with nonimmune IgG or anti-Id. A reduction product of 20 kD was the only monocyte and the major U937 cell polypeptide detected by the anti-Id (Fig. 8). When radioautography was extended from 21 h to 4 d, an additional monocyte polypeptide of 95 kD was detected. Regardless of exposure time, blots probed with nonimmune rabbit IgG were always negative (data not shown).

Discussion

The present studies demonstrate the molecular nature of β-glucan receptors on human mononuclear phagocytes and are the first to characterize the structure of these biochemical entities. The anti-idiotypic antibody, previously shown to bind to and block function of human monocyte β-glucan receptors (17), provided a means to identify and isolate receptors which

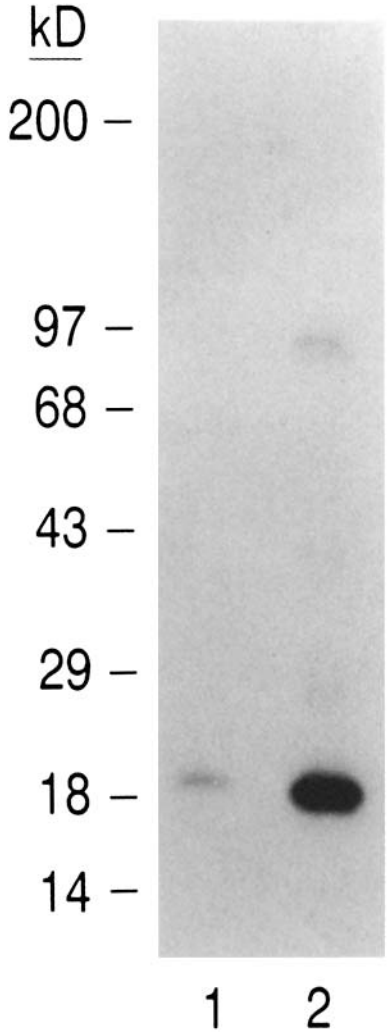

Figure 8. Composition of human monocyte and U937 cell β-glucan receptors. Reduced samples containing 0.4 μg of affinity-purified monocyte (lane *1*) and 0.8 μg of purified U937 cell (lane *2*) proteins were resolved by SDS-PAGE and analyzed in immunoblots probed with the anti-Id, as described in Fig. 6. The radioautograph was developed after 21 h of exposure. A monocyte polypeptide of 95 kD was the only additional species detectable in films exposed for 4 d; control blots revealed no molecules with specificity for nonimmune rabbit IgG or ^{125}I-goat anti-rabbit F(ab')$_2$. The data are representative of three analyses.

initiate phagocytosis of particulate yeast glucan. The availability of a human myelomonocytic cell line provided an alternative to obtaining the large numbers of peripheral blood monocytes required to carry out detailed molecular studies of β-glucan receptors. U937 cells (25) were found to be a suitable cultured source of cells that expressed surface materials antigenic for the anti-Id but not for the corresponding anti-isotype present in the same rabbit antisera before purification of the anti-Id (text). Uptake of radiolabeled anti-Id was saturable at levels of 93–95% by unlabeled anti-Id but was unaffected by the same inputs of nonimmune IgG (Fig. 1). Calculations based on the amounts of IgG specifically bound revealed that 2.6–5.2 × 10^6 constitutive surface molecules were present on each U937 cell; these had an apparent affinity of 1.9 × 10^7 M^{-1} for the anti-Id. Even when consideration was given to these values being derived for logarithmically growing leukemic cells, the data indicated an unexpectedly high number of receptors.

Examination of surface-radioiodinated U937 cells demonstrated that the anti-Id epitope was found on two plasma membrane proteins of 180 and 160 kD (Fig. 2 *A*). Both of these proteins disappeared with reduction and five dominant reduction products of 95, 88, 60, 27, and 20 kD (Fig. 2 *B*, lane *2*) were present. Under reducing and nonreducing conditions, the only other radiolabeled proteins detected were two minor constituents. Neither of these was dependent on the specificity of the anti-Id, as shown by their binding to nonimmune IgG (Fig. 2 *B*, lane *1*). The larger protein of 72 kD was probably the high affinity IgG FcR I (26) and the smaller one of 40 kD was, in all likelihood, cytoskeletal actin nonspecifically bound to IgG (27). Analysis of total U937 cell protein failed to identify additional molecules reactive with the anti-Id (Fig. 3). The 180- and 160-kD proteins, which were both complexes of several disulfide-linked poly-peptides, accounted for 85–90% of the protein purified by affinity column chromatography; the remainder was nearly equally divided among protein aggregates of at least two sizes and a 60-kD protein. The two major proteins were reduced to five polypeptides of 95, 88, 60, 27, and 20 kD and these accounted for 95% of the total sample.

Immunoblots of column purified (Fig. 4) and immunoadsorbed (Fig. 7 *B*) materials indicated the anti-Id epitope to be more prevalent on the 160-kD than on the 180-kD protein. Immunoblots bearing larger amounts of column-purified (Fig. 8, lane *2*) and immunoadsorbed (Fig. 6, lane *3*) materials indicated that each of the five reduction products expressed the anti-Id epitope, with the epitope density always being significantly higher for the 20-kD polypeptide. Elution of immunoadsorbed materials with hot SDS was more efficient in removing firmly bound molecules from solid-phase beads, as evidenced by the small amounts of H and L chains of IgG and a prominent 40-kD band which was likely a dimer of the 20-kD (Fig. 6, lane *3*).

The identification of the U937 cell as a cell type having β-glucan receptors was determined by first incubating detergent-soluble proteins with glucan particles and then detecting glucan-bound proteins by immunoblotting with the anti-Id. The glucan-bound proteins were virtually identical to the proteins adsorbed and immunochemically detected with the anti-Id. The glucan-derived samples contained a dominant 160-kD protein, a minor 180-kD species, and two apparent aggregates. All of these disappeared with reduction and a 20-kD subunit presented as the reduced molecule with the greatest immunoreactivity (Fig. 6, lanes *2* and *4*). For immunoblots bearing nearly equal amounts of detectable antibody- and glucan-derived protein, the anti-Id was most reactive with the 160-kD proteins in both types of samples (Fig. 7 *B*) and, in each case, with the 20-kD reduction product (data not shown). That the glucan-derived proteins were β-glucan receptors of the U937 cells was further supported by immunoblotting eluates of buffer-treated glucan particles

which, despite the efficient removal of bound materials, contained no proteins reactive with the anti-Id.

U937 cells share many surface characteristics with normal human monocytes including structurally equivalent forms of several ligand-specific receptors: IgG FcR I (CD64) and II (CD32), which are both single-chain molecules (26); two species of heterodimeric fibronectin receptors (28), one of which has been shown with monocytes to be identical to the fibroblast receptor (very late antigen 5) (29); and three noncovalent heterodimers of the leukocyte adhesion family (23), Lymphocyte function-associated antigen 1 (CD11a), Mac-1/Mol (CD11b), and p150,95 (CD11c), which share a common β-subunit (CD18). Data obtained from the current studies of U937 cell β-glucan receptors were strikingly similar to monocyte proteins immunopurified with the anti-Id. Detergent-soluble monocyte proteins which were immunoadsorbed and subsequently characterized with the anti-Id contained a major protein of 160 kD, a minor molecule of 180 kD, and two minor apparent aggregates (Fig. 7 *A*, lane *2*). Each of these molecules was composed of several disulfide-bridged polypeptides, of which the predominant immunoreactive species was a 20-kD polypeptide (Fig. 8, lane *1*).

A number of well-studied membrane receptors in cells other than mononuclear phagocytes are complexes of several polypeptides. These include the basophil/mast cell high affinity IgE FcR I (30), the platelet integrin glycoprotein IIb/IIIa complex (CD41) (31, 32), and the TCR/CD3 complex (33), a member of the Ig superfamily (34). In the current studies with U937 cells, blots of the two-dimensional gels probed with the anti-Id clearly showed the 180- and 160-kD proteins to express a common reactivity due to the presence of the disulfide-linked 20-kD polypeptide (Fig. 5). These β-glucan receptors with ligand specificity for microbial carbohydrates may be complex receptors similar to those of unrelated cell types. The production of mAb to restricted epitopes on individual β-glucan receptor protein components and identification of their encoding genes will permit further characterization of the β-glucan receptors, their relatedness to other receptors, and evaluation of their tissue and cellular distribution.

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