Hemonecin (HN) is a bone marrow (BM) protein that promotes specific attachment of immature granulocytes and their precursors within the BM. We report that HN is a glycoprotein containing both mannose and galactose residues, and provide evidence that these carbohydrates mediate granulocytic cell adhesion to HN. Carbohydrate structure was determined by digoxigenin-conjugated lectin binding to HN and indicated the presence of mannose, galactose, sialic acid, and the absence of fucose-linked oligosaccharides. The role of carbohydrates in mediating cell adhesion was examined by chemical and enzymatic deglycosylation. Deglycosylation of HN with trifluoromethanesulfonic acid, which cleaves N- and O-linked oligosaccharides, inhibits 66% of cell attachment to HN, and results in an apparent decrease in molecular weight from 60 to 50 kD. Enzymatic deglycosylation with endo-B-N-acetylglicosaminidase H, which hydrolyzes specific N-linked mannose residues, inhibits 30% of cell adhesion to HN. Finally, the role of these specific sugars in hemonecin-mediated cell adhesion was confirmed with neoglycoprotein blocking. Preincubation of BM cells with mannosyl- and galactosyl-BSA probes produces a dose-dependent inhibition of cell attachment to HN, whereas fucosyl-BSA does not inhibit cell adhesion to HN. These results show that mannosyl and galactosyl partially mediate adhesion of BM granulocytes to HN.

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MATERIALS AND METHODS

Preparation of hemonecin. BM was obtained from rabbit bones and prepared as previously described by Campbell and Wicha. Briefly, frozen rabbit femoral marrow was collected by scraping, then washed with a 3.4 mol/L sodium chloride and TRIS base (Sigma Chemical Co, St. Louis, MO) buffer (pH 7.2) for 3 to 4 days, followed by extraction with 4 mol/L guanidine HCl and dialyzed in Spectropor (Spectrum Laboratories, Houston, TX) dialysis tubing (molecular weight cut-off of 12 to 14 kD). Dialysis was against a 6 mol/L urea and 0.05 mol/L sodium acetate buffer (pH 7.4) and performed overnight at 4°C. The dialysate was applied to a diethyl aminemethyl (DEAE)-Sephacel column (2 × 10 cm) (Bio-Rad, Richmond, CA) and eluted at 0.01 to 0.3 mol/L sodium chloride gradient (pH 7.4). HN eluted from the DEAE column predominantly as a single peak. Fractions eluted from the DEAE-Sephacel containing greater than 50% HN were dialyzed in 0.1 mol/L sodium acetate (pH 7.4) overnight at 4°C. Purity was determined by Western analysis of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) resolved尼trocellulose-transferred proteins probed with an anti-HN monoclonal antibody (MoAb) specific for HN. The dialyzed solution was then applied to a hydroxyapatite column (1 × 15 cm) (Sigma Chemical Co) and the fractions containing unbound HN were collected and applied to an anti-HN MoAb affinity column (Affi-Gel; Bio-Rad). The HN affinity column (0.5 × 5 cm) was prepared by incubating the anti-HN MoAb with the Affi-Gel column bed overnight at 4°C in HEPES buffer (pH 7.0). The HN affinity column was washed with 0.1 mol/L sodium acetate (pH 7.4) and HN eluted with 0.5 mol/L sodium acetate (pH 2.5). The eluate was collected in 1-mL fractions, dialyzed in 0.001 mol/L phosphate-buffered saline (PBS) and concentrated to a volume of 300 to 500 μL.

Glycoconjugate analysis. Digoxigenin-conjugated lectins used to probe ni trocellulose-transferred HN were Gauchnus nivalis agglutinin (GNA); Sambucus nigra agglutinin (SNA); Maakia amurensis agglutinin (MAA); and Alevri aurantia agglutinin (AAA) (Boehringer-Mannheim, Indianapolis, IN).1-5 Carbohydrate specificities were determined by chemical and enzymatic deglycosylation. Deglycosylation of HN with trifluoromethanesulfonic acid, which cleaves N- and O-linked oligosaccharides, inhibits 66% of cell attachment to HN, and results in an apparent decrease in molecular weight from 60 to 50 kD. Enzymatic deglycosylation with endo-B-N-acetylglicosaminidase H, which hydrolyzes specific N-linked mannose residues, inhibits 30% of cell adhesion to HN. Finally, the role of these specific sugars in hemonecin-mediated cell adhesion was confirmed with neoglycoprotein blocking. Preincubation of BM cells with mannosyl- and galactosyl-BSA probes produces a dose-dependent inhibition of cell attachment to HN, whereas fucosyl-BSA does not inhibit cell adhesion to HN. These results show that mannosyl and galactosyl partially mediate adhesion of BM granulocytes to HN.
of these lectins are shown in Table 1. Conjugation of the lectins with digoxigenin enables immunologic detection after recognition of the carbohydrate linkage of the glycoprotein. The antidigoxigenin alkaline phosphatase-conjugated secondary antibody was developed with nitroblue tetrazolium (NBT) and X-phosphate (Boehringer-Mannheim). Positive identification of carbohydrate specificities was confirmed by the control proteins carboxypeptidase Y, fetuin, transferrin, and lactoferrin.

Chemical deglycosylation with trifluoromethanesulfonic acid (TFMS). Purified HN (100 µg) was lyophilized overnight to dryness. Deglycosylation was then performed on a dry-ice ethanol bath using 15 µL of anhydrous TFMS (Aldrich, Milwaukee, WI) at -70°C. The samples were incubated in an ice water bath for 1 hour in sealed test tubes, mixing occasionally, then neutralized by gradually adding 50 µL of pyridine -20°C (Sigma Chemical Co.), diluted with 1 mL of PBS, and then dialyzed at 4°C against several changes of 0.1 mol/L PBS. The samples were pooled, concentrated, and protein determination assays were performed (Bio-Rad). In Western blots 20 µg of protein was loaded onto each lane. Deglycosylated HN was then separated by SDS-PAGE and transferred to nitrocellulose or diluted for attachment assays.

Enzymatic deglycosylation with endo-B-N-acetylglucosaminidase H (Endo-H). Lyophilized Endo-H (0.1 U) (Boehringer-Mannheim) was reconstituted in 200 µL of 0.1 mol/L citrate buffer (pH 5.0), containing 2 mmol/L phenylmethysulfonyl fluoride (PMSF) (Sigma Chemical Co.). Next, 80 µL of 0.3 mol/L citrate buffer (pH 5.0) and 20 µL of 0.1 mol/L citrate buffer (pH 5.0) containing PMSF with or without 10 µmol of Endo-H was added to 100 µg purified HN. The samples were incubated for 16 hours at 37°C, then diluted to 20 µg/mL and the pH adjusted to 7.4 with 0.1 N NaOH. Deglycosylated HN was then separated by SDS-PAGE and transferred to nitrocellulose or diluted for attachment assays.

PAGE. SDS-PAGE 10% gels were prepared according to Laemmli. 19 HN and molecular-weight standards were run under reducing conditions (2-mercaptoethanol), and the protein bands were visualized by silver staining. 20 SDS-PAGE resolved proteins were electrophoretically transferred to nitrocellulose 21 and Western analysis performed using a 1:100 dilution of anti-HN MoAb followed by goat-antimouse IgG alkaline phosphatase-conjugated secondary antibody diluted 1:1,000 (Southern Biotechnology Associates Inc., Birmingham, AL). Separately, samples were probed using 1:1,000 dilution of anti-HN polyclonal antiserum (pAb) followed by rabbit-anti-goat IgG alkaline phosphatase-conjugated secondary antibody diluted 1:1,000 (Cappell Organon Teknika, West Chester, PA). Characterization and dilution titers for primary antibodies were established with HN controls to standardize for antibody concentrations. Alkaline phosphatase-conjugated secondary antibodies were developed with 5-bromo-4-chloro-3-indoyl-phosphate (BCIP) and NBT enzyme substrate system (GIBCO-BRL, Grand Island, NY).

Murine BM cell adhesion assays. BM cells were obtained from femora and tibiae of 12- to 16-week-old C57BL6 mice. Cells were collected by flushing the marrow cavity with serum-free Dulbecco’s modified Eagle’s medium (DMEM) using a 23-gauge needle and a 3-mL syringe. Cells were then dispersed by repeated passage through a 23-gauge needle. Cells were washed in serum-free and methionine/cysteine-free DMEM (ICN Biomedicals, Irvine, CA) and suspended at 5 x 10⁶ cells/mL for 35S labeling. Cells were incubated in methionine/cysteine-free media for 1 hour to deplete endogenous methionine stores and subsequently incubated with 35S-methionine (ICN Biomedicals) for 3 hours at 37°C to the approximate activity of 5 cpm/cell. Radiolabeled cells were diluted with serum-free and methionine/cysteine-free DMEM then incubated on the Immulon 4 plates (Dynatech, Chantilly, VA) at 5 x 10⁶ cells/well to determine adhesion of unfraccionated BM cells to HN.

Purified HN was diluted to 10 µg/mL with PBS. Gelatin (Eastman Kodak, Rochester, NY) was reconstituted with PBS at the same concentration to serve as a negative control protein. Separately, TFMS- or Endo-H-tREATED HN was dialyzed against PBS as described after deglycosylation and diluted to 10 µg/mL. Individual proteins were incubated at 100 µL/well overnight at 4°C on 96-well plates. The plates were then blocked with 5% (vol/vol) milk proteins in PBS for 30 minutes. Radiolabeled cells were incubated on the plates for 1 hour. The nonadherent cells were removed by washing three times with PBS. The adherent cells were solubilized in 1% (vol/vol) Triton X-100, 0.5% (vol/vol) deoxycholate, 5 mmol/L EDTA, 250 mmol/L NaCl, 25 mmol/L TRIS-HCl, (pH 7.4) and quantitated in a TM (Elk Grove, IL) Analytic Liquid Scintillation Counter.

To determine the ability of anti-HN pAb or MoAb to alter attachment of cells to deglycosylated HN, 5 µg/well of anti-HN IgG antisera or anti-HN MoAb were added to TFMS- or Endo-H-treated HN-coated plates for 1 hour before incubation with the radiolabeled cells. Separately, mannose, galactose, and fucose conjugated with BSA probes (Sigma Chemical Co) were added individually and in combination to the cells during the final hour of radiolabeling with 35S methionine. The cells were gently washed in serum-free and methionine/cysteine free media before incubation on HN-coated plates. Pretreatment of unfractionated murine BM cells with 0.1 mol/L mannosyl-BSA, galactosyl-BSA, fucosyl-BSA, or BSA alone for more than 4 hours at 37°C produced no detectable toxicity because trypan blue exclusion by these cells exceeded 98%.

RESULTS

Digoxigenin-conjugated lectin analysis of HN. To identify the carbohydrate residues on HN involved in cytoadhesive interactions with granulocytic precursors, we used digoxigenin-conjugated lectin binding to probe HN transferred to nitrocellulose (Lectin Carbohydrate Specificities shown in Table 1). The presence of terminal mannose residues was indicated by GNA lectin binding to native HN (Fig 1, lane 1), whereas SNA lectin binding indicated the presence of terminal mannose residues, and sialic acid linked to galactose (Fig 1, lane 5). MAA lectin binding to native HN indicated steric acid linked α(2→6) to galactose (Fig 1, lane 5). MAA lectin binding to native HN indicated steric acid linked α(2→3) to galactose (Fig 1, lane 9). The presence of terminally linked fucose residues was investigated with the AAA lectin. The lack of AAA lectin binding to native HN indicates the absence of terminal-linked fucose residues (Fig 1, lane 13). Control proteins of bovine serum albumin, fetuin, and lactoferrin were included to show the specificity of lectin binding (Fig 1; lanes 4, 8, 12, and 14). Thus, as determined by digoxigenin-conjugated lectin binding, HN contains terminal mannose residues, and sialic acid linked α(2→6) and α(2→3) to galactose.

Chemical and enzymatic deglycosylation of HN. The role of carbohydrate in cell adhesion to HN was studied via deglycosylation. We first deglycosylated HN with TFMS to

<table>
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<tr>
<th>Table 1. Lectin Carbohydrate Specificities</th>
<th>Lectin</th>
<th>Specificity</th>
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<tr>
<td>Galanthus nivalis (GNA)</td>
<td>Man α1→6 Man</td>
<td></td>
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<tr>
<td>Sambucus nigra (SNA)</td>
<td>Neu NAc α2→6 Gal</td>
<td></td>
</tr>
<tr>
<td>Maackia amurensis (MMA)</td>
<td>Neu NAc α2→6 Gal</td>
<td></td>
</tr>
<tr>
<td>Alevia aurantia (AAA)</td>
<td>Fuc α1→6 GlcNAc</td>
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HEMONECTIN GLYCOSYLATION AND CELL ATTACHMENT

Fig 1. Lectin binding to native and deglycosylated HN. Purified HN was deglycosylated with TFMS or Endo-H, transferred to nitrocellulose, and probed with indicated digoxigenin-conjugated lectin; GNA (lanes 1 through 4), SNA (lanes 5 through 8), MAA (lanes 9 through 12), and AAA (lanes 13 and 14). Lanes 1, 5, 9, and 13, native HN; lanes 2, 6, and 10, TFMS-treated HN; lanes 3, 7, and 11, Endo-H–treated HN. Positive control proteins, carboxypeptides Y, lane 4; transferrin, lane 8; fetuin, lane 12; lactoferrin, lane 14.

remove N- and O-linked oligosaccharides. This treatment effectively removed carbohydrates as determined by the lack of GNA, SNA, and MAA lectin binding to TFMS-treated HN (Fig 1; lanes 2, 6, and 10). Furthermore, treatment with TFMS results in an apparent IO-kD molecular weight reduction from a relative molecular weight of 60 kD to 50 kD as determined by SDS-PAGE (Fig 2). Chemically deglycosylated HN remains immunoreactive with the anti-HN polyclonal antibody, but does not react with the HN MoAb (Fig 2, lanes 4 and 6). Because TFMS effectively removes carbohydrates, this shows that the polyclonal antibody recognizes epitopes within the polypeptide chain of HN.

We next examined the effects of enzymatic deglycosylation of HN by treating with Endo-H, an exoglycosidase that removes mannose residues, but not complex oligosaccharides. The successful enzymatic removal of N-linked mannose was confirmed by the absence of GNA lectin binding to Endo-H–treated HN (Fig 1, lane 3). In contrast, SNA and MAA lectin binding to Endo-H–treated HN indicated that complex oligosaccharides containing sialic acid and galactose remained intact (Fig 1, lanes 7 and 11). Endo-H–treated HN was detected by polyclonal but not MoAb on Western blot (data not shown).

Role of glycoconjugates in cell adhesion to HN. The role of HN glycoconjugates in hematopoietic cell adhesion was investigated with three alternative approaches: deglycosylation, antibody inhibition, and neoglycoprotein competition.

Cytoadhesion assays were performed under serum-free conditions on HN- or gelatin-coated plates as previously described.3 Fifty-four percent of unfractionated BM cells attached to HN, compared with 3% to gelatin (Fig 3). As we have previously reported,3 greater than 90% of cells adhering to native HN are of the granulocytic lineage as determined by hematoxylin and eosin (H&E) staining (data not shown). The addition of exogenous BSA alone had no effect on cell adhesion to HN. The removal of mannose from HN with Endo-H reduced cell binding by 30%, whereas more complete deglycosylation with TFMS reduced cell attachment by 66%.

Fig 2. Effect of deglycosylation on molecular weight and antibody reactivity. Native or TFMS deglycosylated HN was analyzed by SDS-PAGE and silver staining (lanes 1 and 2) or Western blot using anti-HN polyclonal antiserum (lanes 3 and 4) or anti-HN MoAb (lanes 5 and 6). Native HN, lanes 1, 3, and 5; TFMS-treated HN, lanes 2, 4, and 6. Molecular-weight standard indicated by arrow.

Fig 3. Effect of deglycosylation and anti-HN antibodies on cell adhesion to HN. BM cells were incubated on native or deglycosylated HN-coated wells in the presence or absence of anti-HN polyclonal or MoAb. Cell binding to native HN in the absence of antibody represented 54% of input cells and was normalized to 100%. Cell binding to Endo-H–treated HN was normalized to cells incubated under identical conditions in the absence of enzyme. Lanes 1 through 3, native HN; lanes 4 through 6, Endo-H–treated HN; lanes 7 through 9, TFMS-treated HN; lanes 1, 4, and 7, absence of antibody; lanes 2, 5, and 8, in presence of anti-HN MoAb; lanes 3, 6, and 9, in presence of HN polyclonal antibody; lane 10, gelatin control. Data expressed as mean ± SEM (n = 9 to 12).
Cell adhesion to HN is also inhibited by the anti-HN monoclonal and polyclonal antibodies. Treatment with anti-HN MoAb results in a 30% inhibition of cell adhesion to native HN, whereas the anti-HN polyclonal antibody inhibits greater than 60% of cell adhesion to hemogetin (Fig 3, columns 2 and 3). The addition of anti-HN polyclonal antibody to Endo-H-treated HN reduces binding by an additional 35%, whereas it virtually eliminates cell binding to TFMS-deglycosylated HN (Fig 3, columns 6 and 9). In sharp contrast, the addition of anti-HN MoAb to TFMS- or Endo-H-deglycosylated HN has no effect on cell attachment (Fig 3, columns 5 and 8). Because the MoAb does not react with chemically or enzymatically deglycosylated HN, this suggests that carbohydrates, including mannose, play a role in cell adhesion to HN and suggests that the inhibition of cell attachment by the MoAb results from carbohydrate interactions. Considering that the polyclonal antibody has epitopes within the polypeptide chain of HN and blocks residual cell attachment to TFMS-deglycosylated HN, this suggests a noncarbohydrate component to granulocyte cell adhesion to HN.

The above studies suggest that mannose and galactose residues are present on HN and that these carbohydrates play a role in mediating BM cell attachment. To determine the specificity of these interactions and to provide further evidence for the role of carbohydrates in the mechanism of BM cell binding to HN, we used neoglycoproteins as competitive probes. Preincubation of BM cells with mannosyl- or galactosyl-BSA inhibited subsequent cell attachment to HN (Fig 4). Confirming and extending the deglycosylation studies, cell adhesion to HN was inhibited in a dose-dependent manner with 50% inhibition of cell adhesion occurring at 1 mmol/L mannosyl- or galactosyl-BSA. Pretreatment of BM cells with 0.1 mol/L of mannosyl- or galactosyl-BSA resulted in greater than 90% inhibition of the attachment to HN. In contrast, fucosyl-BSA did not significantly inhibit cell adhesion to HN (Figure 4). The presence of mannosyl- and galactosyl-BSA together resulted in no further inhibition of cell attachment (data not shown).

**DISCUSSION**

We have previously shown that HN is a BM cytoadhesive molecule which shows selectivity for cells of the granulocytic lineage. We used lectin-binding, chemical and enzymatic deglycosylation, and neoglycoprotein competition to investigate the mechanism of granulocyte adhesion to HN. These studies indicate that HN is a glycoprotein and that the interactions between granulocytic precursor cells and HN are mediated by mannose- and galactose-binding lectins on the hematopoietic cells. This interaction was confirmed by both enzymatic/chemical deglycosylation studies as well as competitive inhibition using neoglycoproteins. Thus, attachment of immature granulocytes to HN is significantly inhibited by both chemical removal of N- and O-linked carbohydrates from HN, as well as by enzymatic removal of mannose residues. Confirming these deglycosylation studies, cell adhesion to HN also is markedly reduced by competitive inhibition using neoglycoprotein probes comprised of mannosyl- or galactosyl-BSA. Finally, cell binding is blocked by both polyclonal and monoclonal antibodies to this protein.

The chemical removal (with TFMS) of carbohydrates from HN significantly inhibits cell adhesion. Previous studies indicate that TFMS-mediated deglycosylation effectively removes more than 90% of N- and O-linked oligosaccharides without physiochemical, biological, or immunologic damage to the polypeptide chain. Our data show that polyclonal antibodies to HN retained reactivity with deglycosylated HN, thus confirming the immunologic integrity of the polypeptide core of this protein after deglycosylation. These data also indicate that the decrease in cell adhesion to HN results from TFMS removal of carbohydrates and not the degradation of the polypeptide backbone of this protein.

We next investigated whether specific carbohydrates mediate the adhesive interaction between BM cells and HN. Enzymatic cleavage of mannose (with Endo-H) reduces, but does not abolish, cell adhesion. This shows that this residue is involved in HN cytoadhesive interactions, but is not the solitary component of cell attachment. Because Endo-H specifically cleaves mannose residues without hydrolyzing other N-linked sugars, we used digoxigenin-conjugated lectin binding to show that galactose and sialic acid were still present on Endo-H-treated HN, and that only mannose had been removed. As an alternative to deglycosylation, neoglycoprotein probes (ie, monosaccharides covalently linked to BSA) were used to identify the role of individual carbohydrates in cell attachment. Such reagents allow the study of specific cell-carbohydrate interactions without effecting the integrity of the HN molecule. Confirming the studies with chemically and enzymatically deglycosylated HN, both mannosyl- and galactosyl-BSA inhibit cell binding to HN in...
a dose-dependent manner. Additionally, cell adhesion to HN is not altered by fucosyl-BSA probe, or BSA alone, thus showing the specificity of the mannosyl- and galactosyl-mediated granulocyte interaction with HN.

To provide further evidence for the role of carbohydrates in HN adhesion, both polyclonal and monoclonal antibodies to HN were generated. These antibodies were characterized as to their carbohydrate recognition specificity, and subsequently used to inhibit attachment to native and deglycosylated HN. We first isolated a monoclonal anti-HN antibody that recognizes the mannosic residues on HN (as shown by the fact that this antibody detects native HN, but not Endo-

H-cleaved HN). Confirming the studies of enzymatically cleaved HN, this MoAb only partially blocks cell binding to native HN. We also developed a polyclonal anti-HN antibody that recognizes both native HN and the (chemically and enzymatically) deglycosylated protein. The detection of chemically deglycosylated HN indicates that the polyclonal antibody recognizes epitopes within the protein core. Importantly, polyclonal anti-HN antibody effectively blocks cell binding to both native and deglycosylated HN. In the case of the latter, the polyclonal antibody reduces attachment to background levels. Although much of the reactivity of the polyclonal antibody is lost after deglycosylation of HN by TFMS, the further reduction in cell attachment by this antibody indicates that the protein core of HN plays a role in cell attachment.

We conclude that granulocyte attachment to HN involves both mannose and galactose residues, thus implicating a granulocyte cell-surface lectin in this process. Moreover, the residual binding to chemically deglycosylated HN, and its inhibition by the polyclonal antibody, shows that the protein core of HN also is involved in cell attachment. The small amount of residual activity indicates that the polyclonal antibody recognizes epitope(s) in the core protein. Given the observation that carbohydrate and noncarbohydrate receptor mechanisms mediate cell adhesion to HN, it is likely that different HN receptors exist on granulocytes. This is similar to other matrix molecules. Both fibronectin and thrombospondin contain multiple binding domains for various types of cells although neither interacts with hematopoietic cells via carbohydrate residues. However, our data do not address the nature of the receptor(s) mediating granulocytic cell attachment to HN. Clearly, the majority of HN adhesion is carbohydrate based, and as such would appear to interact with putative lectin(s) on the cell surface. Similar lectin interactions are involved in lymphocyte recognition of the surface of endothelial cells during lymphocyte trafficking. For example, mannose and fucose are capable of blocking the attachment of lymphocytes to endothelial cells in vitro, whereas most other monosaccharides actually increase lymphocyte binding. The binding of granulocytes to HN may use similar mechanisms, although this remains to be proved.

An alternative explanation of the observation that deglycosylation of HN partially inhibits cell binding is that different populations of BM cells attach to the carbohydrate or protein component of HN. However, we have found that greater than 90% of the BM cells that bind to native HN are of the granulocytic lineage. Furthermore, we have found similar effects of deglycosylation of HN on the binding of HL-60 cells (data not shown) as we have for BM cells. These observations suggest that the effects of deglycosylation of HN on cell attachment are not caused by selective effects on different cell populations.

The tissue specific expression of HN in the BM may also relate to progenitor cell localization within the hematopoietic microenvironment. We previously showed that granulocyte progenitor cells bind to HN. Interestingly, Aizawa and Tannoso1 have shown that progenitor cell attachment to marrow stromal cells is mediated by a recognition system involving mannone and galactose. Thus, deglycosylation of stromal cells by galactosidase, and mannosidase abrogated progenitor cell adhesion in these cultures, suggesting a role for these glycoconjugates in mediating cell-cell interactions. Similarly, pretreatment of marrow cells with galactosyl- or mannosyl-neoglycoproteins partially reduced stem cell homing in a murine transplantation model. The greater reduction in binding of granulocytes by mannosyl-BSA then by enzymatic deglycosylation or antibody blocking may result from steric effects of the large mannosyl-BSA conjugate as has been previously reported. These are similar residues to those on HN that we have shown to be involved in granulocyte adhesion. We are currently investigating the role of HN glycosylation in progenitor and stem cell adhesion to HN.

We conclude that the adhesion of BM granulocytes and their precursor cells to HN is mediated by two distinct mechanisms. The first and predominant mechanism of attachment involves the binding of HN galactose and mannose residues to cell-surface receptors (lectins) on the granulocytic cells. In addition, a second component of this adhesion is mediated by interaction between the cell and protein core of HN. The exact nature of these two receptors on granulocytes must await their isolation and cloning.

REFERENCES

15. Wang WC, Cummings RD: The immobilized leukoagglutinin from the seeds of Maackia Amurensis binds with high affinity to complex-type Asn-linked oligosaccharides containing terminal sialic acid-linked alpha-2,3 to penultimate galactose residues. J Biol Chem 263:4576, 1988