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Sequence and expression of a membrane-associated C-type lectin that exhibits CD4-independent binding of human immunodeficiency virus envelope glycoprotein gp120

(virus receptor/type II membrane protein/mannose binding)

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ABSTRACT **The binding of the human immunodeficiency virus (HIV) envelope glycoprotein gp120 to the cell surface receptor CD4 has been considered a primary determinant of viral tropism. A number of cell types, however, can be infected by the virus, or bind gp120, in the absence of CD4 expression. Human placenta was identified as a tissue that binds gp120 in a CD4-independent manner. A placental cDNA library was screened by expression cloning and a cDNA (clone 11) encoding a gp120-binding protein unrelated to CD4 was isolated. The 1.3-kilobase cDNA predicts a protein of 404 amino acids with a calculated M_r of 45,775 and organized into three domains: an N-terminal cytoplasmic and hydrophobic region, a set of seven complete and one incomplete tandem repeat, and a C-terminal domain with homology to C-type (calcium-dependent) lectins. A type II membrane orientation (N-terminal cytoplasmic) is predicted both by the cDNA sequence and by the reactivity of C-terminal peptide-specific antiserum with the surface of clone 11 transfected cells. Native and recombinant gp120 and whole virus bind transfected cells. gp120 binding is high affinity (K_d, 1.3–1.6 nM) and inhibited by mannan, D-mannose, and L-fucose; once bound, gp120 is internalized rapidly. Collectively, these data demonstrate that the gp120-binding protein is a membrane-associated mannose-binding lectin. Proteins of this type may play an important role in the CD4-independent association of HIV with cells.**

One of the first steps in the infection of T cells with human immunodeficiency virus (HIV) is binding of the envelope glycoprotein gp120 to the differentiation antigen CD4 (see ref. 1 for review). The observation of HIV infection of (2–7), and gp120 binding to (8), a number of cell types in the absence of detectable CD4 expression suggests that CD4-independent mechanisms of viral entry also exist. This apparent absence of a strict requirement for CD4 potentially broadens the tissue tropism of the virus. In addition, direct infection by HIV may not always be required to elicit cytopathic effects. For example, CD4-independent binding can occur in neural tissue (8, 9), and exposure of neuronal cultures to gp120 can result in cytotoxicity (9, 10).

The identification of non-CD4 HIV receptors is important if the diverse clinical manifestations observed in HIV infection are to be understood. In this report we describe the use of a eukaryotic expression system (11, 12) to screen cDNAs derived from human placenta, a tissue that exhibits CD4-independent binding of gp120. A cDNA clone was isolated that encodes a gp120-binding protein distinct from CD4. This protein has structural features and binding characteristics that indicate it is a member of the family of C-type mannose-binding proteins.‡

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MATERIALS AND METHODS

Expression Cloning. Pools of 90,000 cDNAs from a placental pCDM8 library (a gift from B. Seed, Harvard Medical School) were transfected by electroporation into COS-7 cells. After 3 days, transfected cells were screened for binding with 1 nM ^{125}I-labeled recombinant vaccinia virus-derived gp120 (vgp120) (refs. 8, 11–13; A. Blomstedt, S. Olofsson, E. Sjogren-Jansson, S. Jeansson, L. Akerblom, J.-E. S. Hansen, and S.-L. Hu, personal communication) after a 1-hr preincubation with CD4a antibody G17-2 (5 μg/ml) by visual inspection of single cells after autoradiography (3-day exposure). [Antibody G17-2 belongs to the CD4a subgroup of CD4 antibodies that block both gp120 binding to CD4 and viral infection (15).] After ≈30 pools had been screened a positive pool was identified and rescreened as successively smaller pools to yield a single cDNA (clone 11). Cells expressing CD4 were obtained following transfection with an equal amount of πH3MCD4 (16). Specificity to gp120 binding was assigned by binding of gp120 purified from HIV_{BRU} (native gp120, ngp120) (17), block of binding by baculovirus-derived gp120 (bgp120) (American Biotechnologies, Columbia, MD), and elimination of binding by immunoprecipitation of the ^{125}I-labeled gp120 preparation with the anti-gp120 monoclonal antibody 110.1 (15), anti-mouse IgG, and protein A-Sepharose. Untransfected COS cells did not display a density of silver grains greater than the background.

Sequencing and Analysis. Clone 11 cDNA in pCDM8 was sequenced on both strands by the dideoxy chain-termination method. Hydropathy was assigned by a Kyte–Doolittle plot (7-residue window) obtained with the Wisconsin Genetics Computer Group package, and sequence alignments and ALIGN scores were generated using PC/GENE.

Ligand Binding, Inhibition, and Internalization Assays. Binding assays were conducted essentially as described (12). For inhibition assays, transfected COS cells or gp120 was preincubated for 1 hr with inhibitor. In ligand internalization assays, transfected COS cells were incubated with 1 nM ^{125}I-gp120 for 5 hr at 4°C, washed, and incubated at 37°C for the time indicated; surface and internalized gp120 were separated by acid treatment (18).

Stable Transfection of HeLa Cells. Clone 11 cDNA was inserted in the *Hin*dIII/*Not* I sites of pcDNAI/Neo vector (Invitrogen, San Diego). HeLa cells were transfected by a calcium phosphate procedure and, after 3 days, selected with Geneticin (GIBCO). Resistant cells were initially enriched for

Abbreviations: HIV, human immunodeficiency virus; vgp120, vaccinia virus-derived gp120; bgp120, baculovirus-derived gp120; ngp120, native gp120.

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‡The sequence reported in this paper has been deposited in the GenBank data base (accession no. M98457).

expression of the gp120-binding protein by two rounds of sterile sorting on a Coulter flow cytometer following staining with fluorescein-labeled bgp120 (American Biotechnologies). Subsequent selection used vgp120 labeled by incubation with the anti-gp120 monoclonal antibody 110-4 (15), followed by a fluorescein-labeled anti-mouse Ig reagent. In total, four cycles of selection were used. Expression of gp120-binding protein over time was followed by staining with vgp120 (100 nM), antibody 110-4, and fluorescein-conjugated anti-mouse reagents. vgp120 binding was inhibited by preincubating cells with mannan (2–4 mg/ml; Sigma) for 30 min.

Generation of Rabbit Antisera. A peptide (564A) corresponding to the C terminus of the polypeptide encoded by clone 11 cDNA (Cys^{384}–Ala^{404}) was conjugated to ovalbumin and used to immunize rabbits. Sera were used following a second booster injection, and titers in peptide ELISAs exceeded 500,000.

RESULTS

cDNA Library Screening. Human placental membranes were found to bind recombinant vgp120 in the presence of antibodies that efficiently block gp120 association with CD4. Fifty to 90% of placental gp120 binding was estimated to be non-CD4. To attempt to identify the protein responsible, a placental cDNA library in the vector pCDM8 was screened by expression cloning procedures. COS cells were transfected with pools of cDNAs and CD4-independent gp120 binding activity was detected with radiolabeled vgp120 in the presence of the CD4a antibody G17-2, which blocks binding of gp120 to CD4. After ≈30 pools had been screened, a positive pool was identified and rescreened as successively smaller pools to yield a single cDNA (clone 11).

Affinity and Characteristics of gp120 Binding. Scatchard plots of gp120 binding to COS cells transfected with clone 11 cDNA gave a K_d of 1.7 ± 0.4 nM (n = 4) for vgp120 and 1.8 ± 0.2 nM (n = 4) for ngp120 (Fig. 1*A*), similar to the results obtained with isolated placental membranes (K_d = 1.3 nM) in the presence of CD4a antibodies (Fig. 1*A*). Calculations from the association and dissociation rate constants gave a similar comparative result. Concurrent analysis of gp120 binding to CD4 expressed on COS cells gave a K_d of 4–5 nM in agreement with previous reports (15, 19). Binding of vgp120 to clone 11 transfected cells was inhibited by bgp120 and ngp120 isolated from purified HIV_{BRU}. Undisrupted psoralen/UV-inactivated HIV_{BRU} also bound clone 11 transfected cells in a gp120-dependent manner (data not shown).

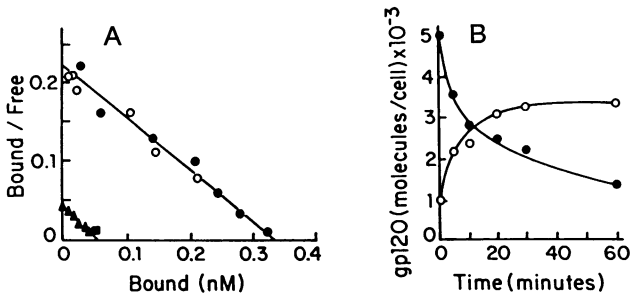

FIG. 1. Characterization of gp120 binding. (*A*) Scatchard analysis of ^{125}I-gp120 binding. ▲, vgp120 binding to placenta (K_d, 1.3 nM; B_{max}, 19 fmol/mg of protein); ■, with CD4a antibody (5 μg/ml); ○, vgp120 binding to clone 11 COS cells (K_d, 1.5 nM; B_{max}, 150,000 receptors per cell); ●, ngp120 (K_d, 1.6 nM; 149,000 receptors per cell). (*B*) Internalization of gp120 by clone 11-expressing COS cells. Points represent the mean of two experiments with vgp120 and ngp120. Transfected COS cells were incubated with ^{125}I-gp120 for 5 hr at 4°C prior to acid stripping procedures, which were conducted at 37°C. ●, Surface; ○, internal.

The fate of gp120 bound to the surface of transfected COS cells was also examined. In these experiments, ^{125}I-gp120 was incubated with cells for 5 hr at 4°C and internalization of the bound gp120 at 37°C was determined using acid stripping procedures (18) to remove cell-surface ^{125}I-gp120. The gp120 was rapidly converted to an acid-resistant form at 37°C (Fig. 1*B*), consistent with the ability of the gp120-binding protein to mediate ligand internalization into the cell.

Predicted Structure of gp120-Binding Protein. The 1.3-kilobase clone 11 cDNA encodes a protein of 404 amino acids with a calculated M_r of 45,775 (Fig. 2). No signal sequence is apparent, but a 21-residue hydrophobic tract (Gly^{41}–Ser^{61}) is present 40 residues from the N terminus (Fig. 3*A*). These features suggest a type II membrane orientation (N-terminal cytoplasmic), which is also supported by the distribution of positively charged amino acids within 15 residues of the hydrophobic region ["positive-inside" rule (20)]. A series of seven complete and one incomplete tandem repeat (Ile^{77}–Val^{249}) of nearly identical sequence follows. The remaining sequence, Cys^{253}–Ala^{404}, shows homology to C-type lectins (Fig. 3*B*): chick hepatic lectin (21), low-affinity IgE receptor (22), the asialoglycoprotein receptors [human H1 and H2 (23) are shown], the rat Kupffer cell receptor (24), and the human macrophage mannose receptor (25, 26).

Binding Inhibition Studies. The sequence homology of the gp120-binding protein to C-type lectins prompted evaluation of the role of sugars in recognition of gp120. Inhibition by a series of saccharides is shown in Fig. 4. Galactose and *N*-acetylgalactosamine did not block gp120 binding to clone 11-expressing COS cells. Mannan was the most potent inhibitor (IC_{50}, 6 μg/ml), followed by L-fucose (K_i, 6 mM); α-methyl D-mannoside (K_i, 15 mM), D-mannose (K_i, 23 mM); and *N*-acetylglucosamine (K_i, 70 mM). Human IgE, sialic acid, and mannose 6-phosphate had no effect on binding. As expected for a C-type lectin, the binding of gp120 to clone 11 required calcium and was blocked by EGTA (K_i, 0.3 mM). None of these sugars affected gp120 binding to CD4. Immune serum from an HIV-infected donor did block gp120/CD4 binding but not binding associated with the gp120-binding protein (data not shown).

Membrane Expression and Orientation of the gp120-Binding Protein. To provide additional evidence for the type II membrane orientation predicted by the cDNA sequence, the gp120-binding protein was expressed in HeLa cells by transfection with clone 11 cDNA ligated in the vector pcDNAI/Neo. After Geneticin selection, a high-binding population was enriched for by sterile sorting on a flow cytometer following staining with directly or indirectly fluorescein-conjugated gp120. Repeated sterile sorting after culture expansion resulted in a population of cells showing stable expression of the gp120-binding activity and with a growth phenotype indistinguishable from the parental, untransfected line. No evidence of cell aggregation was found, suggesting that the expressed lectin was not recognizing glycoproteins resident on the surface of adjacent HeLa cells. Following extended passage, the cells still bound high levels of vgp120 in a mannan-inhibitable manner (Fig. 5*A*).

Immunoprecipitation analyses revealed that the gp120-binding protein expressed on the transfected HeLa cell surface had a molecular mass of ≈46 kDa (data not shown), consistent with size predicted from the cDNA sequence. Flow cytometry studies using rabbit antiserum to the C-terminal peptide 564A confirmed the type II cell surface orientation of the gp120-binding protein on the transfected HeLa cells (Fig. 5*B*). No staining was seen with preimmune serum or with untransfected HeLa cells.

DISCUSSION

A placental library was chosen as the source of cDNA for screening by expression cloning because placental membranes, like neural tissue, bind gp120 in a CD4-independent manner.

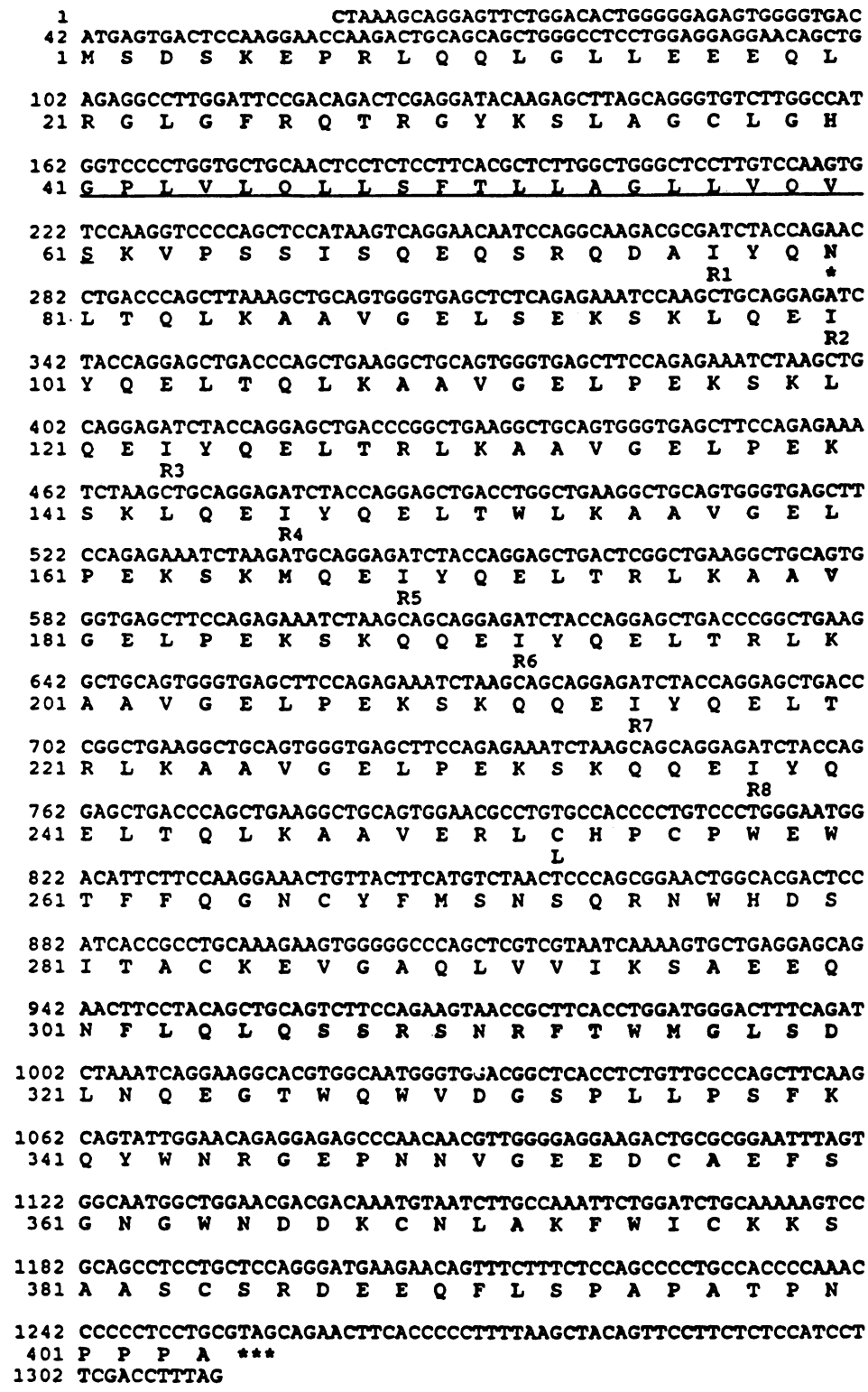

FIG. 2. Nucleotide and deduced amino acid sequence of clone 11 cDNA. The nucleotide sequence preceding the first ATG agrees with the Kozak consensus for translation initiation. The membrane-spanning sequence is underlined and the potential N-linked glycosylation site is marked by a star. The starts of the seven complete and eighth partial repeat (R1–R8) and the beginning of the lectin domain (L) are indicated.

The cDNA isolated encodes a 404-amino acid protein organized into three distinct domains. The sequence predicts a type II membrane orientation (N-terminal cytoplasmic) as suggested by the apparent absence of a signal sequence and the presence of a hydrophobic stop/transfer or anchor sequence (Gly^{41}–Ser^{61}) in the first domain. Application of the "positive-inside rule" (20) for the sequence within 15 residues of the hydrophobic region also predicts a cytoplasmic N terminus in agreement with the homology to membrane-associated C-type lectins with similar membrane orientation (27) (Fig. 3*B*). In addition, the reactivity with stably transfected cells of antiserum to the C-terminal peptide 564A supports this orientation (Fig. 5*B*).

The second domain consists of seven complete and one partial tandem repeat. Circular dichroism spectra in 40% trifluoroethanol of a consensus repeat peptide beginning with

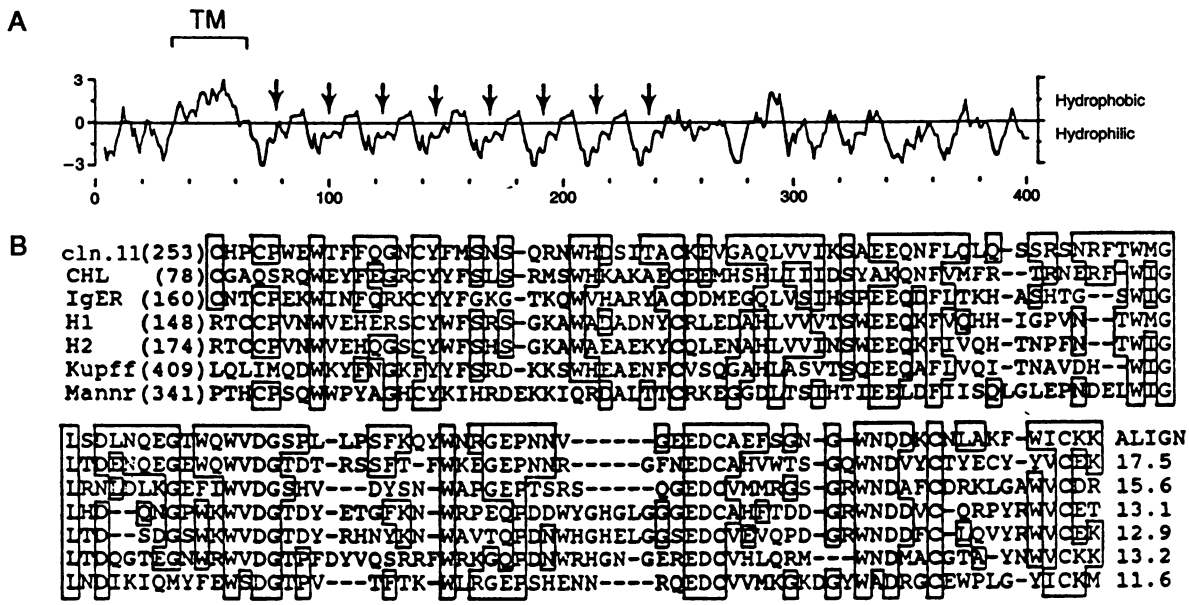

FIG. 3. (*A*) Hydropathicity plot. The predicted transmembrane segment (TM) is bracketed and the starts of the eight amphipathic repeats are indicated by arrows. (*B*) Amino acid alignment of the clone 11 C-type lectin domain. Residues identical to the gp120-binding protein (clone 11) are boxed. ALIGN scores > 3.0 indicate significant sequence similarity. CHL, chicken hepatic lectin; IgER, low-affinity IgE receptor; H1 and H2, human asialoglycoprotein receptors; Kupff, rat Kupffer cell receptor; Mannr, human macrophage mannose receptor.

the β-turn PEKSKLQEIYQELTQLKAAVGEL (single-letter amino acid code) demonstrated an all-α-helical structure (data not shown). Homology to other repeat domains suggested possible tertiary structures including antiparallel helix bundles or a multimeric parallel helix bundle, which would function as spacers to separate the lectin domain from the membrane.

The third domain shows homology to other C-type lectins and contains the conserved motif Trp-Asn-Asp, typical of this group (25). As shown in Fig. 3*B*, the most closely related sequences were the group of type II membrane protein C-type lectins: chick hepatic lectin (21), low-affinity IgE receptor (22), the asialoglycoprotein receptors (23), and the rat Kupffer cell receptor (24). The most similar mannose-binding lectin was one of the eight carbohydrate-recognition domains of the human macrophage mannose receptor (25, 26).

Despite the higher homology to lectins that bind terminal galactose and *N*-acetylglucosamine/galactosamine (27), inhibition studies using sugars and purified gp120 suggest that the terminal mannose residues of high-mannose chains are the primary determinants of binding. For these experiments three forms of gp120 were used: bgp120, which contains only high-mannose structures (28), and vgp120 and ngp120, which contain high-mannose and complex forms (29–31). All three forms have terminal mannose residues in common and all bound with similar affinity (Fig. 1*A*).

A number of studies have pointed to the importance of HIV envelope oligosaccharide side chains (32–35) and, specifically, mannose residues (36–38) in viral infectivity and syncytium formation. The high-affinity recognition of these residues by cell-associated mannose-binding lectins also predicts that such side chains may play a significant role in CD4-independent gp120 binding. Since the affinity of the mannose-binding protein for gp120 exceeds that of CD4, lectins of this type would be effective competitors for gp120 and viral binding on those cells that also express CD4.

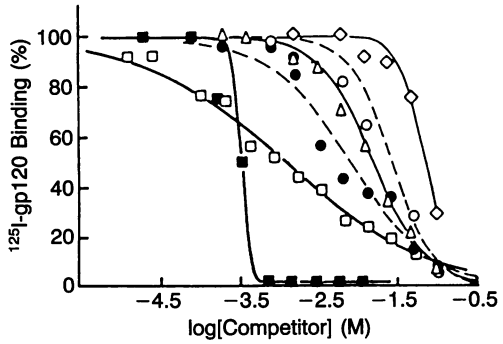

FIG. 4. Inhibition of gp120 binding to COS cells expressing the gp120-binding protein. Both ngp120 (open symbols) and vgp120 (filled symbols) were used and the relative values were the same with both forms of gp120. Mannan concentration is expressed as mg/ml. □, Mannan (IC_{50}, 6 μg/ml); ●, L-fucose (K_i, 6 mM); △, α-methyl D-mannoside (K_i, 15 mM); ○, D-mannose (K_i, 23 mM); ◇, *N*-acetylglucosamine (K_i, 70 mM); ■, EGTA (K_i, 0.3 mM).

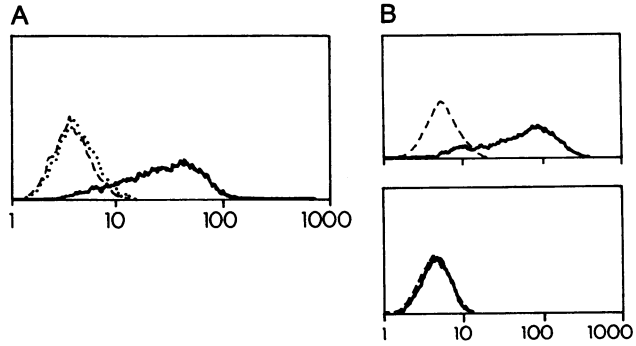

FIG. 5. Stable expression of gp120-binding protein on HeLa cells. HeLa cells were transfected with pcDNAI/Neo vector containing clone 11 cDNA, selected with Geneticin, and sterile-sorted by flow cytometry to enrich for high expression. (*A*) Flow cytometric analysis of transfected HeLa cells incubated with vgp120 (100 nM) (——), with buffer (- - -), or with mannan (4 mg/ml) followed by vgp120 (100 nM) (· · · ·). vgp120 binding was detected by antibody 110-4 followed by a fluorescein-labeled anti-mouse Ig reagent. (*B*) Reactivity of clone 11-transfected (*Upper*) or control (*Lower*) HeLa cells with rabbit preimmune serum (- - -) or antiserum to peptide 564A (——). Ordinate, cell number per channel; abscissa, log green-channel fluorescence.

Both the mannose-specific plant lectins (32, 34, 36, 38) and the human serum 32-kDa mannose-binding protein (39) can inhibit infection of T cells by HIV by a mechanism that does not appear to substantially disrupt gp120/CD4 interactions (38, 40). The consequences of virus binding to a membrane-associated mannose-binding protein are not known, however, and could include CD4-independent infection, as has been suggested in macrophages (40), or entry of the virus into an endosomal pathway and inactivation in the lysosomal compartment, or as seen in epithelial cells, transcytosis (14, 41). Preliminary HIV infection studies on clone 11 transfected HeLa cells are consistent with a role of this lectin in virus binding and internalization, but not infection of these cells (data not shown).

Mannose-binding proteins appear to be able to discriminate between the carbohydrate structures present on gp120 and those present on the surface of normal cells. Since glycosylation of gp120 is directed by host cellular enzymes, this suggests that control of normal cellular glycosylation mechanisms is disrupted by HIV infection. The ability to differentiate viral from host cell oligosaccharides raises the possibility of a therapeutic role for mannose-binding proteins in HIV infection.

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1. Sattentau, Q. J. & Weiss, R. A. (1988) *Cell* **52,** 631–633.
2. Clapham, P. R., Weber, J. N., Whitby, D., McIntosh, K., Dalgleish, A. G., Maddon, P. J., Deen, K. C., Sweet, R. W. & Weiss, R. A. (1989) *Nature (London)* **337,** 368–370.
3. Harouse, J. M., Kunsch, C., Hartle, H. T., Laughlin, M. A., Hoxie, J. A., Wigdahl, B. & Gonzales-Scarano, F. (1989) *J. Virol.* **63,** 2527–2533.
4. Weber, J., Clapham, P., McKeating, J., Stratton, M., Robey, E. & Weiss, R. (1989) *J. Gen. Virol.* **70,** 2653–2660.
5. Kunsch, C., Hartle, H. T. & Wigdahl, B. (1989) *J. Virol.* **63,** 5054–5061.
6. Cao, Y., Friedman-Kien, A. E., Huang, Y., Li, X. I., Mirabile, M., Moudgil, T., Zucker-Franklin, D. & Ho, D. D. (1990) *J. Virol.* **64,** 2553–2559.
7. Omary, M. B., Brenner, D. A., de Grandpre, L. Y., Roebuck, K. A., Richman, D. D. & Kagnoff, M. F. (1991) *AIDS* **5,** 275–281.
8. Kozlowski, M. R., Sandler, P., Lin, P.-F. & Watson, A. (1991) *Brain Res.* **553,** 300–304.
9. Dreyer, E. B., Kaiser, P. K., Offermann, J. T. & Lipton, S. A. (1990) *Science* **248,** 364–367.
10. Brenneman, D. E., Westbrook, G. L., Fitzgerald, S. P., Ennist, D. L., Elkins, K. L., Ruff, M. R. & Pert, C. B. (1988) *Nature (London)* **335,** 639–642.
11. Gearing, D. P., King, J. A., Gough, N. M. & Nicola, N. A. (1989) *EMBO J.* **8,** 3667–3676.
12. Larsen, A., Davis, T., Curtis, B. M., Gimpel, S., Sims, J. E., Cosman, D., Park, L., Sorenson, E., March, C. J. & Smith, C. A. (1990) *J. Exp. Med.* **172,** 1559–1570.
13. Hansen, J.-E. S., Clausen, H., Hu, S.-L., Nielsen, J. O. & Olofsson, S. *Arch. Virol.*, in press.
14. Bourinbaiar, A. S. & Phillips, D. M. (1991) *J. Acquired Immune Defic. Syndr.* **4,** 56–63.
15. Linsley, P. S., Ledbetter, J. A., Kinney-Thomas, E. & Hu, S.-L. (1988) *J. Virol.* **62,** 3695–3702.
16. Peterson, A. & Seed, B. (1988) *Cell* **54,** 65–72.
17. Kozlowski, M. R. & Watson, A. (1990) *Antiviral. Chem. Chemother.* **1,** 175–182.
18. Curtis, B. M., Widmer, M. B., deRoos, P. & Qwarnstrom, E. E. (1990) *J. Immunol.* **44,** 1295–1303.
19. Schnittman, S. M., Lane, H. C., Roth, J., Burrows, A., Folks, T. M., Kehrl, J. H., Koenig, S., Berman, P. & Fauci, A. S. (1988) *J. Immunol.* **141,** 4181–4186.
20. von Heijne, G. & Gavel, Y. (1988) *Eur. J. Biochem.* **174,** 671–678.
21. Drickamer, K. (1981) *J. Biol. Chem.* **256,** 5827–5839.
22. Kikutani, H., Inui, S., Sato, R., Barsumian, E. L., Owaki, H., Yamasaki, K., Kaisho, T., Uchibayashi, N., Hardy, R. R., Hirano, T., Tsunasawa, S., Sakiyama, F., Suemura, M. & Kishimoto, T. (1986) *Cell* **47,** 657–665.
23. Spiess, M. & Lodish, H. F. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 6465–6469.
24. Hoyle, G. W. & Hill, R. L. (1988) *J. Biol. Chem.* **263,** 7487–7492.
25. Ezekowitz, R. A. B., Sastry, K., Bailly, P. & Warner, A. (1990) *J. Exp. Med.* **172,** 1785–1794.
26. Taylor, M. E., Conary, J. T., Lennartz, M. R., Stahl, P. D. & Drickamer, K. (1990) *J. Biol. Chem.* **265,** 12156–12162.
27. Drickamer, K. (1989) *Ciba Found. Symp.* **145,** 45–61.
28. Hsieh, P. & Robbins, P. W. (1984) *J. Biol. Chem.* **259,** 2375–2382.
29. Mizuochi, T., Spellman, M. W., Larkin, M., Solomon, J., Basa, L. J. & Feizi, T. (1988) *Biochem. J.* **254,** 599–603.
30. Mizuochi, T., Matthews, T. J., Kato, M., Hamako, J., Titani, K., Solomon, J. & Feizi, T. (1990) *J. Biol. Chem.* **265,** 8519–8524.
31. Geyer, H., Holschbach, C., Hunsmann, G. & Schneider, J. (1988) *J. Biol. Chem.* **263,** 11760–11767.
32. Lifson, J., Coutre, S., Huang, E. & Engleman, E. (1986) *J. Exp. Med.* **164,** 2101–2106.
33. Matthews, T. J., Weinhold, K. J., Lyerly, H. K., Langlois, A. J., Wigzell, H. & Bolognesi, D. P. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 5424–5428.
34. Hansen, J.-E. S., Nielsen, C. M., Nielsen, C., Heegaard, P., Mathiesen, L. R. & Nielsen, J. O. (1989) *AIDS* **3,** 635–641.
35. Fenouillet, E., Gluckman, J. C. & Bahraoui, E. (1990) *J. Virol.* **64,** 2841–2848.
36. Robinson, W. E., Jr., Montefiori, D. C. & Mitchell, W. M. (1987) *AIDS Res. Hum. Retroviruses* **3,** 265–282.
37. Muller, W. E. G., Schroder, H. C., Reuter, P., Maidhof, A., Uhlenbruck, G. & Winkler, I. (1990) *AIDS* **4,** 159–162.
38. Gattegno, L., Ramdani, A., Jouault, T., Saffar, L. & Gluckman, J.-C. (1992) *AIDS Res. Hum. Retroviruses* **8,** 27–37.
39. Ezekowitz, R. A. B., Kuhlman, M., Groopman, J. E. & Byrn, R. A. (1989) *J. Exp. Med.* **169,** 185–196.
40. Larkin, M., Childs, R. A., Matthews, T. J., Thiel, S., Mizuochi, T., Lawson, A. M., Savill, J. S., Haslett, C., Diaz, R. & Feizi, T. (1989) *AIDS* **3,** 793–798.
41. Amerongen, H. M., Weltzin, R., Farnet, C. M., Michetti, P., Haseltine, W. A. & Neutra, M. R. (1991) *J. Acquired Immune Defic. Syndr.* **4,** 760–765.