

### RECOGNITION OF HUMAN IMMUNODEFICIENCY VIRUS GLYCOPROTEINS BY NATURAL ANTI-CARBOHYDRATE ANTIBODIES IN HUMAN SERUM

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**SUMMARY:** Anti-carbohydrate antibodies were isolated from Human immunodeficiency virus (HIV) negative human serum by affinity chromatography using yeast mannan followed by protein A. The purified mannan-binding IgG (MBIgG) bound to HIV glycoproteins gp160, gp120 and gp41 in Western blot. Immunofluorescence revealed that MBIgG bound to HIV/IIIB-infected H9 cells but not to uninfected H9 cells, suggesting that carbohydrate structures recognized by MBIgG are specifically expressed on HIV-infected cells. MBIgG did not neutralize infectivity of HIV. These results show that normal human serum contains natural antibodies reactive to carbohydrate structures of HIV glycoproteins propagated in human cells.

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Human immunodeficiency virus (HIV) is the retrovirus identified as the causative agent of acquired immunodeficiency syndrome (AIDS) (1,2). The first step in HIV infection is binding of the viral envelope glycoprotein gp120 to the CD4 receptor on the cell surface (3). The gp120 molecule is highly glycosylated and studies using lectins (4,5), endoglycosidases (6,7), or oligosaccharide processing inhibitors (8,9) have shown that the carbohydrate moiety of this glycoprotein is involved in the interaction between gp120 and CD4. Blocking or removing the glycans of HIV glycoproteins causes a decrease in the ability of gp120 to bind to CD4 resulting in a decrease in viral infectivity. Recently Hansen et al. (10) demonstrated that some anti-carbohydrate murine monoclonal antibodies inhibit HIV infectivity independent of both virus strain and cell line used for virus propagation. Our goal is to develop human monoclonal antibodies neutralizing diverse HIV strains for passive immunotherapy against AIDS, and these studies suggest that the glycans of HIV glycoproteins might be a target for human monoclonal antibodies.

Several investigators have reported that human sera contain naturally occurring heterophil antibodies capable of recognizing animal oncoviral (retrovirus) envelope glycoproteins and that those antibodies are directed to carbohydrate moieties of viral glycoproteins (11-13). Glycosylation of viral glycoproteins is thought to be a host-dependent process, and therefore, it is expected that glycoproteins of viruses and the host

**Abbreviations:** HIV, Human immunodeficiency virus; MBIgG, mannan-binding IgG; MBP, mannose-binding protein; HIVIG, Human HIV-1 Immune Globulin.

cell show similar glycosylation patterns. Accordingly, some investigators have suspected that human antibodies cannot specifically recognize carbohydrate structures of viral glycoproteins propagated in human cells (11). On the other hand, altered glycosylation in host cells induced by virus infection have been reported (14-16). Prior to this report, it was not known whether human sera contained antibodies recognizing carbohydrate structures of human retroviral glycoproteins such as HIV gp120 and whether those antibodies have any antiviral activities.

To address these questions, we isolated anti-carbohydrate IgG from pooled HIV negative human serum by affinity chromatography using yeast mannan followed by protein A and examined its binding and neutralizing activities against HIV.

## MATERIALS AND METHODS

**Purification of Anti-carbohydrate Human IgG:** Anti-carbohydrate human IgG was partially purified from pooled HIV negative human serum by affinity chromatography using yeast mannan by the method of Summerfield et al. (17). Yeast mannan (Sigma) was immobilized on activated CH-Sepharose 4B (Pharmacia) by the standard method. One hundred ml of normal human serum, which was obtained from Gemini Bioproducts, Inc. (Calabasas, CA) and had been shown anti-HIV antibody-negative by FDA approved methodology, was mixed with an equal volume of 0.02M Tris-HCl, pH7.4 /40mM CaCl<sub>2</sub> /1M NaCl and centrifuged at 4000 x g for 20min. The supernatant was first passed through Sepharose CL-4B (Pharmacia) pre-column (10ml) and then loaded onto the mannan-Sepharose column (10ml) equilibrated with 0.02M Tris-HCl, pH7.4 /20mM CaCl<sub>2</sub> /0.5M NaCl. The column was washed with the same buffer used for equilibration and then washed with 2mM EDTA to elute calcium-dependent mannose-binding proteins (MBP) (17). Calcium-independent mannan-binding proteins, which are principally IgG, were eluted with 3M KSCN. The protein peaks in EDTA and KSCN eluates were collected, concentrated and dialyzed against phosphate-buffered saline (PBS). Mannan-binding IgG (MBIgG) in KSCN eluate was further purified by protein A affinity chromatography. The dialysate of KSCN eluate was mixed with an equal volume of 0.2M sodium phosphate, pH8.0 /10mM EDTA and loaded onto a protein A-Sepharose CL-4B (Pharmacia) column (100ul) equilibrated with 0.1M sodium phosphate, pH8.0 /5mM EDTA. The column was washed with the same buffer used for equilibration and IgG was eluted with 0.1M acetic acid. Eluted IgG was concentrated and dialyzed against PBS. The molecular weights of proteins were determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using a 10% gel based on the Laemmli system (18). IgG concentration was determined by single radial immunodiffusion using anti-human IgG antiserum. The binding of the purified antibodies to mannan was verified by enzyme-linked immunosorbent assay (ELISA) using mannan-coated plates as described previously (17).

**Western Blot Analysis:** Western blot was performed using Du Pont's HIV-1 Western blot IgG kit. Assay procedures were followed as per the directions, except that blotting powder (nonfat dry milk) was not added to blotting buffer because we suspected that the blotting powder might inhibit binding of MBIgG to HIV glycoproteins on the Western blot strips. Human HIV-1 Immune Globulin (HIVIG) and MBIgG were separately allowed to react with the blotted viral antigens at a concentration of 2ug/ml. HIVIG was obtained as a positive control from Dr. Alfred Prince and National Heart, Lung, and Blood Institute through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH.

**Indirect Immunofluorescence:** Uninfected and HIV/IIIB-infected H9 cells which were grown in RPMI 1640 containing 10% fetal calf serum, 2mM L-glutamine and 50ug/ml gentamycin (10% FCS /RPMI) were used. The cells were washed with PBS and dried onto glass slides. The cells on the slides were fixed with acetone at 4°C for 10min. The fixed cells were incubated with 50ug/ml of antibody at room temperature (R.T.) for 1hr. After washing the slide three times in PBS, the cells were incubated with fluorescein isothiocyanate (FITC)-labelled goat anti-human IgG antibody (Tago, Inc., Burlingame,

CA) at R.T. for 1hr mounted with buff  
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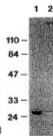


Fig.1. SDS-PAGE using yeast mannan-Sepharose 4B.

Fig.2. Western blot analysis of HIV-1 antigens on Western blot strips from which allowed to react with MBIgG at 1:100.

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CA) at R.T. for 1hr. Finally the slide was washed in PBS as before and a cover slip was mounted with buffered glycerine, and observed on a fluorescence microscope.

**Neutralization Assay:** Culture supernatant of HIV/IIIB-infected H9 cells grown in 10% FCS /RPMI was used as a virus inoculum. Neutralizing activities of antibodies were determined by the method of Montefiori et al. (19), except that MoT cells were used as a target cell. MoT cell is a T cell line transformed by HTLV-II and was kindly provided by Dr. Paul Feorino at CDC in Atlanta.

## RESULTS

**Purification of Anti-carbohydrate Human IgG:** Anti-carbohydrate human IgG was partially purified from pooled HIV negative human serum using a mannan-Sepharose affinity column. Since human serum contains calcium-dependent MBP (17), the column was first washed with 2mM EDTA to remove calcium-dependent lectins. SDS-PAGE revealed that the prominent band in the EDTA eluate from the mannan-Sepharose column was a 30kDa protein (Fig.1), whose size was identical to that of subunit calcium-dependent human MBP (17). Then the column was washed with chaotropic ions (3M KSCN) to elute calcium-independent mannan-binding proteins including IgG. In SDS-PAGE, the main band in the KSCN eluate was a 150kDa protein. This protein was further purified from the KSCN eluate using protein A-Sepharose column, and was identified to be IgG by single radial immunodiffusion (data not shown). The binding of the purified antibodies to mannan was examined by ELISA using mannan-coated plates. The antibodies reacted to

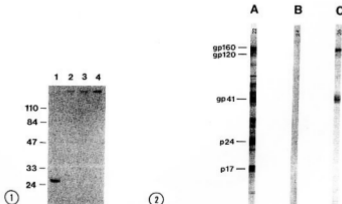


Fig.1. SDS-PAGE analysis of human serum proteins purified by affinity chromatography using yeast mannan followed by protein A. Samples are EDTA eluate (lane 1), KSCN eluate from mannan-Sepharose column (lane 2), eluate from protein A-Sepharose column (lane 3) and standard human IgG (lane 4).

Fig.2. Western blot analysis of HIV antigens recognized by MB1gG. Du Poot's HIV-1 Western blot strips were incubated with HIVIG (A), non-manipulated human serum from which MB1gG was isolated (B) and MB1gG (C). HIVIG and MB1gG were allowed to react at a concentration of 2 $\mu$ g/ml. Human serum was tested at a dilution of 1:100.

mannan in a dose-dependent manner (data not shown). From 100ml of human serum approximately 200ug of MBIgG was recovered.

**Western Blot Analysis:** The ability of MBIgG to bind to HIV was first examined by Western blot. MBIgG bound to HIV envelope glycoproteins gp160, gp120 and gp41 (Fig.2). No binding of MBIgG to non-glycosylated viral proteins, such as p24 and p17, was observed. Non-manipulated human serum from which MBIgG was isolated did not show any bands in Western blot at a final dilution of 1:100.

**Indirect Immunofluorescence:** Immunofluorescence was performed to examine the binding specificity of MBIgG. MBIgG bound to HIV/IIIB-infected H9 cells but not to uninfected H9 cells (Fig.3). This result suggests that the carbohydrate structures recognized by MBIgG are specifically expressed on HIV-infected cells. Although the fluorescence intensity of MBIgG on infected cells was much weaker than the same concentration of HIVIG, we speculate that the binding affinity of MBIgG, which was isolated from non-immunized serum, to HIV glycoproteins is lower than that of HIVIG,

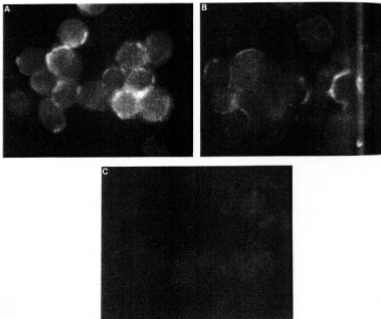


Fig.3. Indirect immunofluorescence of HIV-infected and uninfected cells stained by MBIgG. HIV/IIIB-infected H9 cells (A,B) and uninfected H9 cells (C) were incubated with HIVIG (A) and MBIgG (B,C) followed by FITC-labelled goat anti-human IgG antibody.

which was from immunized serum. Alternatively, the fluorescence of MBIgG was weaker because only a few antibody species of the total MBIgG, which are thought to recognize several carbohydrate structures on mannan, cross-react to carbohydrate moiety of HIV glycoproteins.

**Neutralization Assay:** Since it was shown that MBIgG selectively binds to HIV glycoproteins, its neutralizing activity against HIV was examined. MBIgG did not neutralize HIV/IIIB at concentrations up to 50ug/ml, while HIVIG neutralized about 50% of virus at the same concentration (data not shown).



## DISCUSSION

In this study we have shown that normal human serum contains antibodies capable of recognizing the carbohydrate moiety of HIV envelope glycoproteins. Prior to this paper, several investigators reported the presence of antibodies reactive to the glycans of animal oncoviral envelope glycoproteins in normal human sera (11-13). These antibodies are thought to have been naturally elicited as a result of exposure of various pathogens including bacteria, fungi, and human viruses with envelope glycoproteins such as herpes viruses. It is unlikely that individuals whose sera possess anti-oncovirus antibodies are infected with animal retroviruses such as simian sarcoma-associated virus or murine leukemia virus. Antibodies reactive to HIV glycoproteins described here may also have been elicited naturally rather than as a consequence of HIV exposure.

HIV envelope glycoprotein gp120 is highly glycosylated and high-mannose type glycans represent 50% of total N-glycan in gp120 (20). Human MBP, which is thought to be an acute-phase immunoreactant, selectively binds to HIV-infected cells and inhibits HIV infectivity (21). Hansen et al. (10) have demonstrated that some anti-carbohydrate murine monoclonal antibodies neutralize HIV and these antibodies recognize three different carbohydrate structures (Le<sup>y</sup>, A<sub>1</sub>, and sialyl-Tn) which are not normally found on human lymphocytes but are expressed in lymphocytes chronically infected with HIV. Our results show that MBIgG bound to HIV-infected H9 cells but not to uninfected H9 cells, suggesting that MBIgG recognizes HIV-induced abnormal carbohydrate structures.

The biological significance of natural anti-carbohydrate antibodies in human serum is not clear. Like human MBP, MBIgG may play a role in early host defense. MBIgG did not neutralize HIV infectivity at concentrations up to 50ug/ml, but higher concentrations of MBIgG may be necessary. MBIgG, as well as natural antibodies described by Bendinelli et al. (22), may require complement for virus neutralization. It has been reported that FCS contains a glycoprotein to which natural human antibodies bind (23). In our neutralization assays we used culture supernatant from HIV-infected cells as a virus inoculum, so MBIgG may have been absorbed by bovine glycoproteins from FCS. Although MBIgG did not neutralize HIV, it may induce antibody-dependent cytotoxicity of HIV-infected cells which we did not test.

We have developed several anti-fungal human monoclonal antibodies reactive to mannan and examined their binding ability to HIV glycoproteins by Western blot,

however, they did not bind to HIV (unpublished data). These observations support our speculation that not all antibodies reactive to mannan cross-react to HIV glycoproteins, and also suggest that not all human sera contain natural antibodies reactive to HIV glycoproteins.

Finally, it is expected that other human retroviruses, such as HTLV-I and HTLV-II, may also induce abnormal carbohydrate structures on human cells. It remains to be tested whether MBIgG recognizes envelope glycoproteins of HTLV-I and HTLV-II as well as HIV glycoproteins. Antibody recognizing human retrovirus-induced, group-specific carbohydrate structures might be a good reagent for diseases caused by human retroviruses.

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