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Effect of anti-carbohydrate antibodies on HIV infection in a monocytic cell line (U937)

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Summary

Monoclonal antibodies (mAbs) against carbohydrate epitopes of gp120 have recently been found to inhibit HIV infection of lymphocytes in vitro thereby opening new possibilities for vaccine considerations. Antibody-dependent enhancement of infection has however come increasingly into focus. This study therefore investigated the neutralization of HIV in a monocytic cell line (U937) using mAbs against these carbohydrate gp120-epitopes. While antibodies against one of the epitopes (A_1) neutralized infection of U937 cells despite binding to the Fc-receptor, one mAb against the sialosyl-Tn epitope enhanced infection. This enhancement was independent of complement and could be blocked by mAb Leu3a against the CD4-receptor. The study indicated that enhancement of infection in monocytic cells can occur by the same anti-carbohydrate antibodies that neutralize infection in lymphocytes, and that antibody mediated enhancement may depend on location of the epitope on gp120 rather than whether the antibody binds Fc-receptors.

HIV; U937; Neutralization; Antibody-dependent enhancement; Carbohydrate

Introduction

In HIV-infected patients emphasis has been placed on infection of the CD4+ lymphocyte population as the predominant pathogenetic feature in AIDS is depletion of these lymphocytes (Ho et al., 1987). An important feature

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of HIV infection seems, however, to be infection of monocytes/macrophages. In these cells HIV is not equally cytopathic as in lymphocytes, and infected macrophages may be an important reservoir of HIV (Gendelman et al., 1989; Gartner et al., 1986). Infection of macrophages is believed to involve the same receptors as in lymphocytes, namely the CD4 receptor and LFA-1, which has recently been implicated in the initial adhesion/penetration of HIV in both lymphocytes and monocytes (Valentin et al., 1990; Hansen et al., 1991). If the infection mechanism is identical in the two cell types, antibodies which inhibit HIV infection of lymphocytes should also inhibit infection of macrophages. However, several reports have indicated that in some cases, antibodies against the major envelope glycoprotein of HIV, gp120, may augment CD4-binding and enhance infection of lymphocytes and especially of macrophages (Homsy et al., 1990; Joualt et al., 1989). It is suggested that this phenomenon results from phagocytosis of HIV/antibody-complexes bound to the Fc receptor of macrophages (Takeda et al., 1988; Matsuda et al., 1989; Robinson et al., 1989). If relevant in vivo, this would present a problem in vaccination programs.

Recently we have demonstrated, that antibodies against carbohydrate epitopes (A_1, Le^y and sialosyl-Tn) of the major envelope glycoprotein, gp120, of HIV inhibit infection in lymphocytes (Hansen et al., 1990). These carbohydrate antigens, especially the sialosyl-Tn epitope which shows a very restricted expression in normal human adults (Kjeldsen et al., 1988), could thus have potential as components of an anti-HIV vaccine. We therefore found it important to test the neutralization of HIV by the anti-carbohydrate antibodies in a monocytic in vitro model.

Materials and Methods

Cells and virus

The monocytic cell line U937 (Andersen and Abraham, 1980; Levy et al., 1985) was cultured at 37°C, 5% CO_2 using RPMI 1640 with 10% heat-inactivated Fetal Calf Serum, 2 μM glutamine, 100 IU/ml penicillin, 20 μg/ml gentamicin and 100 IU/ml streptomycin (growth medium). Cells were maintained at a concentration of 2–10 $\times$ 10^5 cells/ml and medium was exchanged twice weekly. The HIV-1 strain SSI-002 was used in all infectivity experiments. This HIV isolate was previously found to infect U937 cells readily (Hansen et al., 1991), and in HIV infection of lymphocytes this isolate was neutralized by the anti-carbohydrate mAbs used in this study (Hansen et al., 1990).

Antibodies

Monoclonal anti-carbohydrate antibodies TKH2 (IgG_1), B72.3 (IgG_1), AH16 (IgG_3) and BM1 (IgM) were purified from mouse hybridoma culture

TABLE 1

Epitope specificity of monoclonal antibodies used in this study

Epitope	Structure	mAb	Reference
Sialosyl-Tn 1988	NeuAcα2→6GalNAcα1→O-Ser/Thr	TKH2 and B72.3 (IgG_1)	Kjeldsen et al., 1988
A_1	GalNAcα1 ↘3 Galβ1→3GlcNAcβ1→R ↗2 Fucα1	AH16 (IgG_3)	Abe et al., 1984
Le^y	Galβ1→4GlcNAcβ1→R ↗2 Fucα1 ↗3 Fucα1	BM1 (IgM)	Abe et al., 1983
Le^x	Galβ1→4GlcNAcβ1→R ↗3 Fucα1	SH1 (IgG_3)	Singhal, unpublished data
Protein	Histo-blood group A transferase	WKH1 (IgG_1)	White et al., 1990

supernatants by protein A Sepharose chromatography or ion-exchange chromatography (BM1). mAbs WKH1 (IgG_1) and SH1 (IgG_3) were used in immunofluorescence assays as dialyzed hybridoma supernatants (for epitope-specificities, see Table 1). mAb Leu3a against the CD4 receptor was from Becton Dickinson, California, and mAb anti-CD18 was from Dakopatts, Glostrup.

All antibody preparations were dialyzed against PBS, filtered (0.22 μm) and stored at −80°C. None of the antibodies influenced cell growth as evaluated by vital staining with trypan blue.

Inhibition assay

Fifteen or one hundred 50% cell culture infectious doses ($CCID_{50}$) HIV-1 isolate SSI-002 were incubated for 1 h in varying amounts of mAb and used to inoculate 2×10^6 U937 cells in 500 μl growth medium for 2 h. In some experiments fresh human serum from a normal donor was used as a source of complement and admixed to the mAb in dilution series from 100 μl serum. After extensive washing, the cells were transferred in quadruplicates to a 24-well cell culture plate (400 000 cells per well in 1 ml growth medium). Cultures were maintained for 14 days at 37°C, 5% CO_2, and 500 μl culture medium was exchanged with fresh medium after 4, 7 and 10 days. In some experiments, untreated virus was used to inoculate cells which had been preincubated with mAb and washed. HIV antigen in culture supernatants was measured by a double sandwich ELISA using purified immunoglobulin from AIDS patients as previously described (Nielsen et al., 1987; Hansen et al., 1990). Antigen concentration was expressed relative to antigen concentration at corresponding

days in control-cultures of untreated cells inoculated with untreated HIV. The ELISA was titrated so that the optical density was in the 0.6 1.0 range in samples from control cultures.

Immunofluorescence assay

One million U937 cells suspended in 50 μl suspension buffer (PBS containing 1% bovine serum albumin and 0.1% azide) were incubated with purified mAb for 30 min at 4 °C.

The mAb-adsorbed cells were washed once in 1 ml cold PBS and incubated for another 30 min at 4°C with fluorescein isothiocyanate-conjugated Fab-rabbit anti-mouse or anti-human immunoglobulin (Dakopatts, Glostrup) diluted 1:30 in suspension buffer. After incubation, the cells were washed once and resuspended in 1 ml PBS with 1% paraformaldehyde. The cells were analyzed by flow cytometry using a fluorescence-activated cell sorter (FACScan, Becton Dickinson, CA). mAbs against CD18 (Dakopatts, Glostrup) and mAbs WKH1 and SH1 were used as controls.

Fc-receptor binding by the labelling mAbs was tested by preincubating cells for 30 min in purified IgG_{1-3} (Sigma, St. Louis, MO) prior to labelling with mAb. And to see whether mAb/HIV complexes could bind to cells, mAb was preincubated with HIV-1 for 30 min before labelling cells with this mixture. In order to test for specificity of TKH2/B72.3 labelling of the cells, these were incubated with 0.1 U/ml neuraminidase type X (Sigma, St. Louis, MO) for 30 min at room temperature prior to labelling with mAbs.

Results

Influence of anti-carbohydrate antibodies on infectivity

A viral inoculum of 15 $CCID_{50}$ HIV-1 isolate SSI-002 was preincubated with 37.5 μg AH16, BM1 or TKH2 prior to infection of a monocytic cell line (U937). Monitoring infection by HIV antigen determination using culture supernatants at days 7, 10 and 14 revealed that mAb AH16, specific for the carbohydrate A_1-antigen (Abe et al., 1984), inhibited HIV infection (Fig. 1). HIV antigen concentration at day 7 was less than 1% of controls. mAb BM1, specific for the carbohydrate Le^y-antigen, did not influence infection of the U937 culture. mAb TKH2, however, enhanced infection as shown by a 2-fold increase of HIV antigen output in the culture medium at day 7 compared to controls. The inhibition or enhancement found with AH16 or THK2 respectively was due to an interaction with the virus and not with the target cells as preincubation of target cells followed by washing prior to inoculation with HIV did not affect infection (Table 2).

The effects of mAbs on HIV infection were dose-dependent (Fig. 2). Thus at the time of maximum effect (day 7), AH16 inhibited HIV infection in a

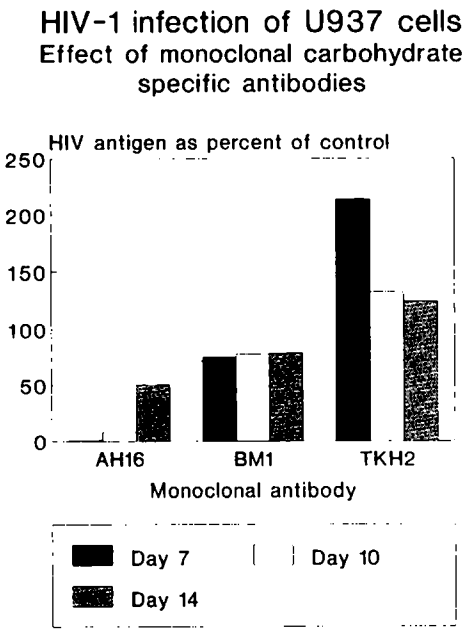

Fig. 1. 2.5 μg/CCID$_{50}$ of three carbohydrate specific mAbs, known to inhibit infection in lymphocytes at this concentration (Hansen et al., 1990), was incubated with 15 CCID$_{50}$ HIV-1 (isolate SSI-002). This was then used to infect 2 × 10^6 U937 cells (monocytic cell line) which were cultured in growth medium without mAb for 14 days. HIV antigen in culture supernatants was measured by ELISA on days 7, 10 and 14. Antigen concentration was expressed relative to antigen concentration at corresponding days in control-cultures inoculated with untreated HIV. Mean of quadruplicate determinations.

concentration dependent manner, and TKH2 enhanced infection also in a concentration dependent manner while BM1 did not have any effect on HIV infection. Using a viral inoculum of 15 CCID$_{50}$ a two-fold increase in HIV antigen production was obtained with 2.5 μg/CCID$_{50}$ TKH2 but with a viral inoculum of 100 CCID$_{50}$, 0.7 μg/CCID$_{50}$ TKH2 was sufficient to double

TABLE 2

Inhibition or enhancement of HIV infection in U937 cells by mAb AH16 or TKH2 was due to interaction of mAbs with virus and not with cells

	Preincubation of	
	HIV	U937
PBS	663 ± 13	647 ± 17
AH16	3 ± 2	628 ± 39
TKH2	1210 ± 38	651 ± 21

Either 2 × 10^6 U937 cells or 15 CCID$_{50}$ HIV-1 were preincubated with 37.5 μg mAb or PBS for 1 h. Cells preincubated with mAb or PBS were washed in PBS and inoculated with 15 CCID$_{50}$ HIV-1 preincubated with PBS or mAb. HIV antigen detection in culture supernatants after 7 days of culture was expressed as mean optical density (490 nm) of quadruplicate experiments (mean ± SEM).

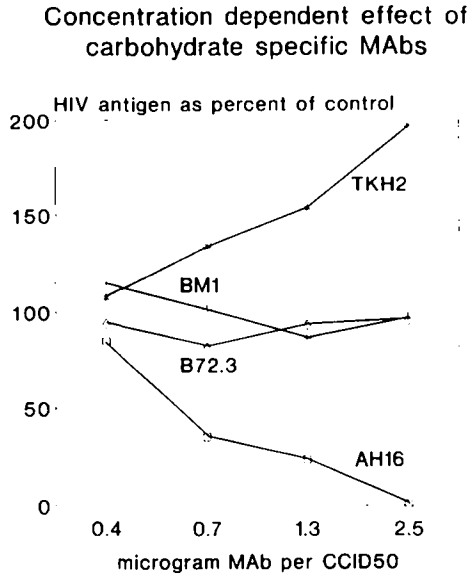

Fig. 2. Dose-response effect of monoclonal carbohydrate-specific antibodies (mAb) on HIV infection of U937 cells. A viral inoculum of 15 $CCID_{50}$ HIV-1 isolate SSI-002 was preincubated with a dilution series of mAb prior to inoculation of U937 cells and subsequent culture. After 7 days, HIV antigen concentrations of culture supernatants were determined and expressed as percent of untreated control cultures. Mean of quadruplicate determinations.

antigen production. The dose-response per $CCID_{50}$ of the inhibitory mAb AH16 did not vary with the viral inoculum. Curiously, mAb B72.3 which is reported to have the same epitope specificity as TKH2 and also the same Ig subtype, neither inhibited nor enhanced infection. The enhancing effect of TKH2 was counteracted by the inhibitory effect of anti-CD4 (Leu3a) as infection of cells preincubated in 0.3 μg Leu3a was reduced by 80% when inoculated with untreated HIV and by 79% when inoculated with HIV preincubated with 2 $\mu g/CCID_{50}$ TKH2. Finally, to examine the possibility of complement mediated enhancement, a dilution series of fresh human serum from a normal donor was admixed to the mAb prior to inoculation. No alterations of the dose-response profiles of any mAb were observed.

FACS-analysis

Binding of mAbs to the target cells was examined by FACS analysis (Table 3). mAbs AH16 and BM1 bound to U937 cells. mAb BM1 was of IgM-type and labelled 32% of the cells. mAb AH16 was of IgG_3-type and 49% of the cells were labelled by this mAb. However, incubation of the cells with indifferent IgG before labelling with AH16 abrogated AH16 binding to the cells (2%). Staining with the non-neutralizing mAb SH1 (data not shown) of the same Ig-subtype as AH16, also showed some labelling (51%) of the U937

TABLE 3

Immunofluorescence staining of U937 cells with mAbs against carbohydrate epitopes using FACS-analysis

mAb	PBS	IgG	Neuraminidase
AH16 (IgG_3)	49	2	ND
TKH2 (IgG_1)	8	7	3
B72.3 (IgG_1)	6	2	5
BM1 (IgM)	32	ND	ND
Leu3a (IgG_1)	57	54	55
anti-CD18 (IgG_1)	100	100	ND
WKH1 (IgG_1)	2	2	ND
SH1 (IgG_3)	51	2	ND
None	3	5	5

Cells were preincubated in PBS, indifferent IgG or neuraminidase prior to labelling. Results, representative of several experiments, indicate percent labelled cells. mAbs WKH1 and SH1 were included in this assay as immunoglobulin-type controls along with monoclonal antibodies against the membrane antigens CD18 and CD4 (Leu3a).

cells which was blocked by preincubation of the cells with indifferent IgG (2%). Allowing AH16 to bind to $HTLV_{IIIB}$ prior to incubating the cells with this mixture, labelled 43% of the cells and also this labelling could be eliminated with indifferent IgG (2%).

Labelling of the U937 cells with TKH2 and B72.3 was only slightly above background levels (Table 3). The apparent reduction in labelling after preincubation of the cells with either IgG or neuraminidase did therefore not conclusively indicate Fc-receptor binding or epitope-specific binding with these mAbs.

Discussion

Four monoclonal antibodies AH16, TKH2, B72.3 and BM1 were tested for inhibition of HIV infection of cells from the monocytic cell line U937. The antigen specificities of these mAbs are well characterized (Table 1). AH16 binds to the blood group carbohydrate antigen A_I (Abe et al., 1984), TKH2 and B72.3 bind to the O-linked carbohydrate antigen sialosyl-Tn (Tettamanti and Pigman, 1968; Kjeldsen et al., 1988) and BM1 binds to the carbohydrate antigen Le^y (Abe et al., 1983). These mAbs have recently been found to precipitate the major HIV envelope glycoprotein gp120 and to inhibit infection of lymphocytes in vitro (Hansen et al., 1990).

mAb AH16 inhibited HIV infection of the monocytic U937 cells as this mAb does in lymphocytes. At the same time AH16, whether complexed with HIV or not, was found to bind to U937 cells. This binding was abrogated by IgG indicating that the binding of AH16 by U937 cells was mediated by binding to Fc-receptors on the cells. mAb TKH2, reacting with a different carbohydrate epitope on gp120, enhanced infection. A lower concentration of TKH2 was

necessary to produce a certain level of enhancement with a high viral inoculum (100 $CCID_{50}$) than with a low viral inoculum (15 $CCID_{50}$), which indicates that with a high inoculum more mAb-virus complexes were formed and were available for infection of the target cells. This enhancement was dependent on the CD4 receptor as Leu3a blocked the enhancing effect of TKH2. mAb B72.3 which is reported to have the same epitope-specificity as TKH2 had no effect on infection. This discrepancy was not due to differences in Ig subtype as both mAbs were IgG_1. We have however observed the same enhancement with IgG from a rabbit immunized with the sialosyl-Tn containing ovine submaxillary mucin (Hansen et al., unpublished data). We suggest that small differences in structure (for instance glycosylation) of the immunoglobulins TKH2 and B72.3 may be responsible for their different effect in a biological assay although their specificities and Ig-subtypes are indistinguishable in chemical assays.

The anti-sialosyl-Tn mAbs labelled a small proportion of the U937 cells. There was some reduction in labelling by prior incubation of the cells with IgG which could indicate Fc-receptor binding. However, also neuraminidase treatment of the cells seemed to reduce labelling indicating an epitope-specific binding. In both cases, the labelling was close to background levels, and it does therefore not seem warranted to make any conclusion about the type of binding involved in the interaction between U937 cells and the anti-sialosyl mAbs.

Enhancement of HIV infection in monocytes is mediated by complement, antibodies to the viral glycoproteins or both and utilizes cellular complement-receptors and Fc-receptors (Reisinger et al., 1990; Robinson et al., 1989; Robinson et al., 1990a; Takeda et al., 1988; Matsuda et al., 1989; Homsy et al., 1990). In most studies, this enhancement has been found to depend also on CD4/gp120 binding. In this study we too found CD4 binding to be necessary for enhancement in the U937 model as the enhancement was blocked by mAb Leu3a against the CD4 receptor. However, using fresh serum as a source of complement we found no indications that the observed enhancement with mAb TKH2 involved complement.

Antibody-dependent HIV infection of CD4-negative fibroblasts has recently been show to occur after induction of cellular Fc receptors by CMV infection (McKeating et al., 1990). And in dengue virus infection, enhancing antibodies seems responsible for aggravated disease in vivo (Kliks et al., 1989). The in vitro phenomenon of antibody dependent enhancement of HIV infection may therefore reflect a serious problem for vaccine development. As this study shows for carbohydrate-specific anti-gp120 antibodies, Fc-receptor binding of antibody/HIV complexes is not sufficient for enhancement. As has also been found for peptide-specific antibodies, the epitope specificity is most important (Robinson et al., 1990b). It therefore seems possible and desirable to map both enhancing carbohydrate and peptide epitopes of the HIV envelope glycoproteins, and to exclude these from HIV vaccines.

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