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ORIGINAL INVESTIGATION

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Inhibition of HIV-1 infection by monoclonal antibodies to carbohydrates of *Schistosoma mansoni*

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Abstract Patients infected with HIV-1 develop a potent humoral immune response against the virus, but HIV-1 primary isolates are remarkably resistant to neutralizing antibodies. Considering that the envelope glycoprotein of HIV-1 (gp120/41) is heavily glycosylated, we investigated whether anti-carbohydrate antibodies could inhibit HIV-1 infection in vitro. We studied the neutralizing activity of three monoclonal antibodies (mAbs) raised to carbohydrates of *Schistosoma mansoni,* against seven primary isolates of HIV-1. Assays were performed infecting peripheral blood mononuclear cells from normal donors with viral isolates previously treated with mAbs. Viral strains used were tropic for the coreceptors CCR5, CXCR4, and dual-tropic ones. We found that the anti-glycan mAbs vigorously inhibited HIV-1 infection, regardless of the preferential coreceptor usage of the isolate, in a dose-response manner. Importantly, five isolates were resistant to neutralization by two HIV-1 antibody-positive human sera endowed with potent anti-HIV-1 inhibitory activity. Our findings suggest that carbohydrates of the HIV-1 viral envelope may be a target of an effective humoral immune response elicited by vaccination.

Keywords HIV-1 · AIDS · Neutralizing antibodies · *Schistosoma mansoni*

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Introduction

The human immunodeficiency virus type 1 (HIV-1), the etiological agent of the acquired immunodeficiency syndrome (AIDS), infects and replicates in cells that present the surface marker CD4 [2]. The entry of HIV-1 into the target cells requires, in association with the CD4 molecule, the simultaneous virus binding to chemokine receptors. Some viruses interact with the β-chemokine receptor CCR5 and are termed R5-tropic isolates, some bind to the α-chemokine receptor CXCR4 and are termed X4-tropic, and others are able to use either one, and are then classified as R5X4 dual-tropic viruses [2]. R5 viruses can be isolated from patients during the whole course of the infection, are usually associated with the asymptomatic clinical status of the HIV-1-infected patients, and are the phenotypes preferentially transmitted in vivo [33]. X4 and R5X4 isolates are more frequently found in patients progressing from the asymptomatic clinical status to AIDS [10].

An immune response against HIV-1 can be detected few weeks after the primary infection [6]. Following the virus seeding in the lymphoid tissues, the viral replication is controlled mainly by cytotoxic $CD8^+$ T cells [3, 20]. Most patients infected by HIV-1 also mount a strong humoral immune response against the virus [6], but, so far, there is no clear evidence showing that the antibodies are really effective in limiting the progression of the infection to AIDS. Several studies have shown that primary viruses are remarkably resistant to neutralization by antibodies, either from vaccinee sera or from serum samples of HIV-1-infected individuals [9]. Likewise, it has been shown that resistance to neutralizing antibodies is independent of the virus preferential coreceptor usage

[7, 18, 21, 27]. Experimental evidence indicates that primary isolates of HIV-1 escape the humoral immune response because immunodominant epitopes on the envelope glycoprotein (gp120/41) of these viruses are not accessible to neutralizing antibodies [4, 25]. In contrast, tissue culture laboratory-adapted (TCLA) isolates of HIV-1 are sensitive to neutralizing antibodies, most likely because immunodominant epitopes are more exposed to immunoglobulin binding [28].

The extensive glycosylation of gp120/41 may hide immunodominant peptidic epitopes, preventing the binding of neutralizing antibodies [23, 32]. However, these carbohydrates may also function as neutralization epitopes, since monoclonal antibodies (mAbs) against oligosaccharides inhibited HIV-1 infection in cell-free virus experiments, as well as in syncytium-inducing assays [13, 14, 24, 26]. In this study we investigated whether mAbs primarily directed to egg carbohydrates of the helminth *Schistosoma mansoni* could neutralize HIV-1 infection mediated by primary, non-TCLA isolates, in vitro. This study was performed taking into account the existence of similarities between the carbohydrates of the HIV-1 gp120/41 and those of *S. mansoni* egg antigen, such as the common presence of the tetrasaccharide Lewis^y [1]. Moreover, the tested anti-*S. mansoni* mAbs react with fucose-containing epitopes [17, 29], and 90% of the gp120/41 glycosylation sites are fucosylated [19]. Our results show that these mAbs vigorously inhibit isolates of HIV-1 characteristically resistant to HIV-1 antibody-positive human sera, regardless the virus preferential coreceptor usage, suggesting that envelope carbohydrates of HIV-1 may be a target of an effective humoral immune response elicited by vaccination.

Material and methods

Cells

Peripheral blood mononuclear cells (PBMCs) from healthy donors were obtained by density gradient centrifugation (Histopaque, Sigma Chemical Co., St. Louis, MO) from buffy coat preparations, and subsequently stimulated for 3 days with 2 μg/ml phytohemagglutinin (PHA, Sigma) in RPMI 1640 medium (Sigma), supplemented with 10% fetal bovine serum (FBS, HyClone, Logan, UT), HEPES, penicillin and streptomycin. For HIV-1 infection assays, PHA-stimulated PBMCs were further cultured in the same medium, containing 5 U/ml recombinant IL-2 (Sigma). The human astroglioma U87 cells stably transfected with CD4 and with CCR5 or with CXCR4 (U87-CD4$^+$CCR5$^+$ and U87-CD4$^+$CXCR4$^+$, respectively) were donated by Dan Littman (Howard Hughes Medical Institute, New York, NY). They were maintained in Dulbecco's minimal essential medium (Sigma) containing 10% FBS, glutamine, penicillin/streptomycin, puromycin (1 μg/ml, Sigma) and geneticin (G418; 300 μg/ml, Sigma), and were split twice a week, as described [15].

Virus isolates and serum samples

The following HIV-1 isolates were used: (1) Ba-L, 168.1, 168.10 and T-CSF (donated by Dr. Michael A. Norcross, CBER, US FDA, Bethesda, MD); (2) 95BRRJ10, 95BRSP01, 95BRSP07 and 95BRBA07, which were isolated in our laboratory, as described [5]. Stock viruses have been kept at –70°C, and expanded only in PBMCs from HIV-1-seronegative blood donors, except T-CSF, which has been expanded in the CD4$^+$ tumor cell line PM-1. The general phenotypic characteristics and the preferential coreceptor usage of the isolates have already been reported by us [11]. In summary, Ba-L and 168.1 are macrophage-tropic, non-syncytium-inducing (NSI) and R5-using viruses; the TCLA virus T-CSF and the primary isolates 95BRRJ10 and 95BRSP01 are X4-tropic, syncytium-inducing (SI) variants; 95BRBA07, 95BRSP07 and 168.10 are R5X4-using, SI isolates. Serum samples from HIV-1-positive individuals and from normal donors were provided by the Brazilian Network for HIV Isolation and Characterization [5], inactivated at 56°C for 30 min, and stored at –70°C until use.

Monoclonal antibodies

The anti-*S. mansoni* carbohydrate mAbs E1, E3 and E5 were obtained in BALB/C mice immunized with either egg or soluble egg antigens of *S. mansoni*, as previously described [17]. mAbs E1 (IgG2b) and E3 (IgG3) recognize oligosaccharide epitopes containing fucose on their structure. mAb E5 (IgM) reacts with the oligosaccharide lacto-*N*-fucopentaose III (LNFPIII), which contains the Lewis^x sugar on its structure. E1 and E5 were purified by protein A or anti-IgM chromatography, then dialyzed against PBS. E3 was salted out of culture supernatant, and then also dialyzed against PBS. The mAbs were filtered before using in the neutralization studies.

Viral neutralization by human sera

HIV-1-positive supernatants (5 ng/ml p24 Ag) were incubated with HIV-1 antibody-positive human serum (RJ31 or SP09), at a final dilution of 1:100, for 60 min at 37°C, and the virus-serum suspension was added to transfected U87 cells previously seeded in 96-well flat-bottom culture plates (1×10^4/well). After overnight incubation, cells were washed, fresh medium was added back and culture was maintained at 37°C, 5% CO_2, for 7–10 days. Viral replication was evaluated by detecting the activity of the enzyme reverse transcriptase (RT) in culture supernatants, as described [16]. HIV-1 antibody-negative human serum was used as a control. Neutralization of R5- and X4-tropic isolates was studied infecting U87-CD4$^+$CCR5$^+$ or U87-CD4$^+$CXCR4$^+$ cells, respectively, and of R5X4-tropic isolates infecting both cells.

Virus neutralization by anti-carbohydrate mAbs

HIV-1-positive cell-free supernatants (5 ng/ml p24 Ag) were incubated with different concentrations of mAbs and, after 1 h at 37°C, the mAb-virus mixture was added to PHA-activated PBMCs in 96-well flat-bottom culture plates (2×10^5/well per 200 μl). Cultures were incubated overnight at 37°C, 5% CO_2, and cells were washed to remove the excess of virus and antibodies. Regular medium with 5 U/ml IL-2 was added back, and cells were cultured for additional 7–10 days. The same procedures were done with irrelevant mAbs as a control, and virus replication was assessed by detecting the RT activity in the culture supernatants [16].

Results

Before addressing the sensitivity of the primary isolates to HIV-1 antibody-positive serum samples or to anti-*S. mansoni* carbohydrate mAbs, we selected human sera presenting a potent neutralizing activity against the HIV-1 TCLA isolate T-CSF, which has been shown to be highly sensitive to antibody neutralization [4]. Several randomly chosen serum samples were tested, and all of

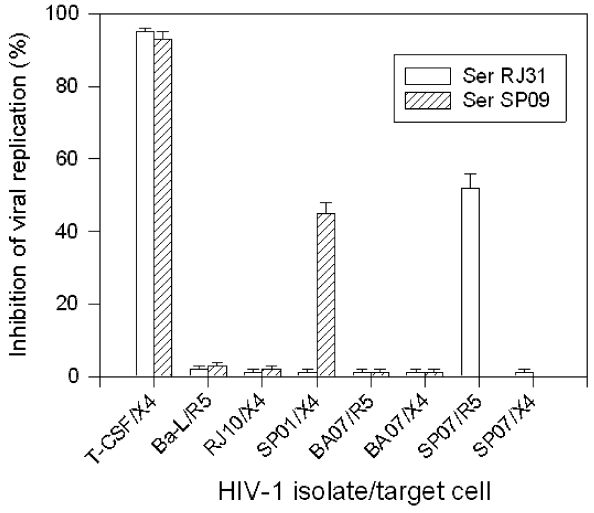

Fig. 1 HIV-1 sensitivity to human sera. U87-transfected cells were exposed to viral supernatants preincubated or not with HIV-1antibody-positive human serum (dilution 1:100). Cells were washed, fresh medium was added back and culture was maintained for 7–10 days. Viral replication was evaluated by detecting the RT activity in culture supernatants, and data represent the means ± SEM of four experiments done in triplicates. R5 and X4 indicate the cells U87-CD4$^+$CCR5$^+$ and U87-CD4$^+$CXCR4$^+$, respectively. HIV-1 antibody-negative human serum did not affect viral replication (*RT* reverse transcriptase)

them readily neutralized this variant (data not shown). Two of these sera (RJ31 and SP09) were eventually selected according to their ability to inhibit at least 90% of T-CSF replication in U87CD4$^+$CXCR4$^+$ cells at dilutions not under 1:100 (Fig. 1).

Subsequently, we analyzed the sensitivity of five primary isolates of HIV-1 to serum samples RJ31 and SP09, in the context of coreceptor usage. As shown in Fig. 1, the isolates Ba-L (R5-tropic), 95BRRJ10 (X4-

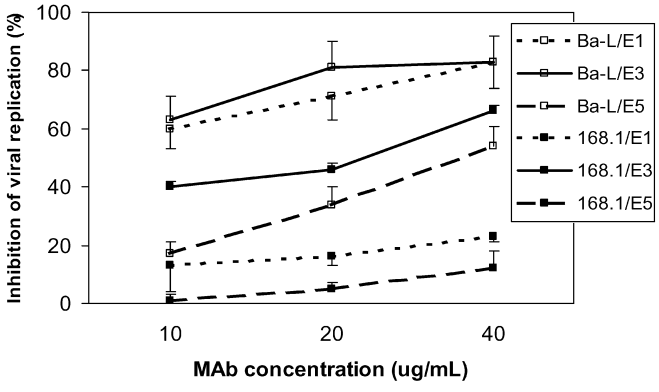

Fig. 2 Inhibition of R5-tropic isolates by anti-carbohydrate mAbs. Peripheral blood mononuclear cells were exposed to viral supernatants preincubated or not with different concentrations of mAbs. Cells were washed, regular medium with 5 U/ml IL-2 was added back, and culture was maintained for 7–10 days. Viral replication was evaluated by detecting the RT activity in culture supernatants, and data represent the means ± SEM of four experiments done in triplicates. Abbreviations following the virus names indicate the mAb used. Irrelevant control mAbs did not affect viral replication

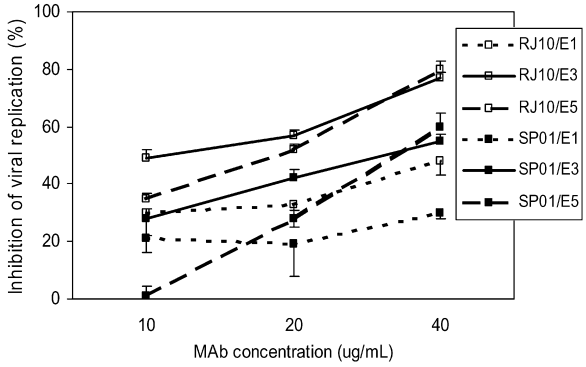

Fig. 3 Inhibition of X4-tropic isolates by anti-carbohydrate mAbs. Legend as for Fig. 2. Virus denominations were shortened for simplification, and abbreviations following the virus names indicate the mAb used

tropic) and 95BRBA07 (X4R5-tropic) were completely resistant to both sera at 1:100 dilution, whereas the isolate 95BRSP01 (X4-tropic) was mildly inhibited by serum SP09. The dual-tropic isolate 95BRSP07 was only moderately inhibited by serum RJ31 in U87CD4$^+$ CCR5$^+$ cells, but it was completely resistant to this serum in infection assays using U87CD4$^+$ CXCR4$^+$ cells (virus 95BRSP07 was not tested with serum SP09).

We next examined the possible neutralizing activity of anti-carbohydrate mAbs against primary isolates of HIV-1 presenting different tropisms for chemokine receptors. In marked contrast with the observed resistance to neutralization by HIV-1 antibody-positive serum samples, the replication of HIV-1 isolates was inhibited by anti-carbohydrate mAbs in a concentration-dependent manner, irrespective of the virus preferential coreceptor usage. Regarding the R5-tropic isolates (Fig. 2), virus Ba-L was highly sensitive to mAbs E1 and E3, with inhibition of infection ranging from 60% to 83% with 10 μg/ml to 40 μg/ml of each mAb, and partially blocked by E5 (54% inhibition with 40 μg/ml). The isolate 168.1 (Fig. 2) was resistant to mAbs E1 and E5, but it was inhibited (66%) by 40 μg/ml of E3. Concerning the neutralization of X4 isolates (Fig. 3), virus 95BRRJ10 was moderately (55%) to strongly (78%) inhibited by 20 μg/ml and 40 μg/ml of mAbs E3

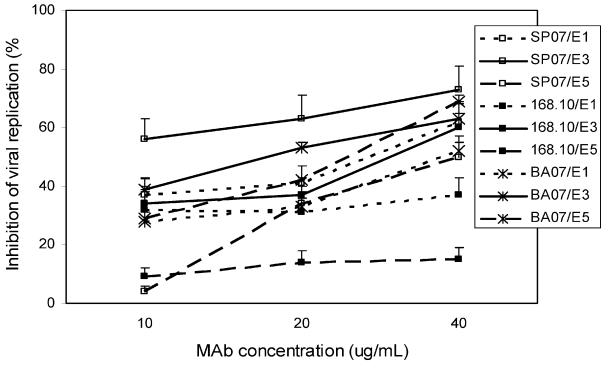

Fig. 4 Inhibition of R5X4-tropic isolates by anti-carbohydrate mAbs (see legend to Fig. 2)

and E5. mAb E1 showed weak or no blocking activity against the X4 virus samples. Relative to R5X4-tropic viruses (Fig. 4), we found that all mAbs neutralized the isolates 95BRSP07 and 95BRBA07 at 40 μg/ml, with levels of inhibition ranging from 50% to 73% and from 50% to 67%, respectively. Isolate 168.10 evaded the neutralizing activity of the mAbs E1 and E5, but it was sensitive to 20 μg/ml (52% inhibition) and 40 μg/ml (66% inhibition) of E3.

Discussion

Primary isolates of HIV-1 are remarkably resistant to antibody neutralization, either to hyperimmune sera from vaccinees or from HIV-1-infected individuals [9]. The antibody resistance exhibited by primary isolates is related to reduced exposure of immunodominant epitopes in the glycoprotein (gp 120/41) of the viral envelope [4, 25, 32]. In this work, we also observed that immune sera of HIV-1-infected individuals could not inhibit the infection of some HIV-1 primary isolates. In agreement with other authors [7, 18, 21, 27], we did not find clear evidence that the preference for using the coreceptors CXCR4 or CCR5 was a critical property determining the level of sensitivity of primary isolates of HIV-1 to neutralization by anti-HIV-1 serum samples (Fig. 1).

Carbohydrates constitute approximately 50% of the gp120/41 mass [19] and may hide antigenic peptide epitopes from antibodies, limiting the protective efficiency of the humoral immune response against HIV-1 [23, 32]. On the other hand, the glycan residues of the viral envelope can function as neutralization sites, as described previously [13, 14, 24, 26]. In this study, we report that anti-carbohydrate mAbs, raised against the egg antigen of *S. mansoni,* could neutralize seven primary isolates of HIV-1, and that the inhibitory activity occurred irrespective of the preferential coreceptor usage of the isolates.

The anti-glycan mAbs clearly inhibited the CCR5-using isolates Ba-L and 168.1 (Fig. 2), the CXCR4-tropic isolates 95BRRJ10 and 95BRSP01 (Fig. 3) and the dual-tropic, CCR5/CXCR4-using viruses 168.10, 95BRSP07 and 95BRBA07 (Fig. 4). The neutralization of these isolates was consistent and reproducible, despite minor variations in the intensity of the inhibition mediated by each mAb, implying that putative antibodies induced by gp120/41 carbohydrate residues may be very effective against HIV-1. Importantly, five isolates (Ba-L, 95BRRJ10, 95BRSP01, 95BRBA07 and 95BRSP07) displayed a marked resistance to human sera endowed with potent anti-HIV-1 activity (Fig. 1).

We have examined whether these anti-glycan mAbs bind to human PBMCs, by FACScan and by Western blot, and we found no binding (preliminary results, not shown). This suggests that the anti-HIV-1 activity may be due to direct binding to the virus, albeit we have not yet performed the binding assays on the virus. It is possible that the mAbs recognize epitopes located near to either CD4 or coreceptor binding sites on the viral envelope. Reactions between the mAbs and the ubiquitous carbohydrate determinants at key locations on gp120/41 could prevent the virion-cell membrane interaction and fusion by steric hindrance, forming a non-specific blockade to infection. Hansen et al. [13] identified four carbohydrate epitopes on HIV-1 envelope that are recognized by neutralizing antibodies, and two of them, Lewis^y (also found in *S. mansoni* egg antigen) and A, have fucose on their structure. We could envisage that the mAbs EI, E3 and E5, which recognize fucose-containing epitopes, inhibit HIV-1 by binding to those determinants and, possibly, to other similar glycan residues on gp120/41.

Our findings, together with those described by other authors [13, 14, 24, 26], suggest that the carbohydrates of the HIV-1 viral envelope may be a target for an effective humoral immune response elicited by vaccination. In fact, rabbits immunized with the mucin-type carbohydrate sialosyl-Tn, which is present on the HIV-1 gp120/41 [14], developed neutralizing antibodies against the laboratory-adapted isolates IIIB and MN [8]. Moreover, the broadly neutralizing human anti-HIV-1 mAb 2G12 recognizes a mannose-dependent epitope, composed primarily of carbohydrates, with no involvement of gp120 peptides [24, 26]. In conclusion, our study contributes to the knowledge concerning the development of reliable immunogens able to elicit a protective humoral immunity against HIV-1. Further studies should address other relevant aspects, such as a comparative analysis of neutralizing potency against different HIV-1 subtypes.

Curiously, recent reports have described that patients infected with HIV-1 and with acute infections by *Orientia tsutsugamuchi* [30], measles [22] or dengue viruses [31] presented a significant decline in the plasma HIV-1 load, and in vitro studies showed that the human herpes virus-6 suppressed HIV-1 replication in lymphoid tissues by inducing the production of β-chemokines [12]. These clinical and experimental findings, together with our present results, may reveal the other side of the coin of the association between HIV-1 and co-pathogens.

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