The *N*-linked glycan of the V3 region of HIV-1 gp120 and CXCR4-dependent multiplication of a human immunodeficiency virus type 1 lymphocyte-tropic variant

Britt Losman[a], Marlene Biller[a], Sigvard Olofsson[a], Kristian Schønning[b], Ole Søgaard Lund[b], Bo Svennerholm[a], John-Erik Stig Hansen[b], Anders Bolmstedt[a,*]

[a] *Department of Clinical Virology, University of Göteborg, Guldhedsgatan 10 B, S-413 46 Göteborg, Sweden*
[b] *Department of Infectious Diseases 144, Hvidovre Hospital, 2650 Hvidovre, Denmark*

Received 29 March 1999; received in revised form 18 May 1999

Abstract We have previously shown that an *N*-glycosylation site of N306 of HIV-1 gp120 is not necessary for the HIV-1 infectivity but protects HIV-1 from neutralising antibodies. In contrast Nakayama et al. [FEBS Lett. (1998) 426, 367–372], using a virus with an identical V3 region, suggested that elimination of this particular glycan reduced the ability of T-tropic HIV to bind to CXCR4 and hence its ability to infect T cell lines. We therefore re-examined the ability of a mutant virus, lacking the N306 glycan, to replicate in various types of cells and found no change in co-receptor usage for mutant virus. The ability of mutant virus to replicate or to induce syncytia in infected cells was similar to that of wild type virus. These results corroborate our original observation, confirming that the induced mutation in the N306 glycosylation site neither impairs nor improves the ability of mutant virus to replicate in permissive cells.

Key words: Acquired immunodeficiency syndrome; U937; Macrophage; Human immunodeficiency virus type 1; Carbohydrate

1. Introduction

The V3 region of the major glycoprotein, gp120, specified by human immunodeficiency virus type 1 (HIV-1) is an important factor in several aspects of the viral pathogenesis. This region constitutes the principal neutralisation domain (PND), harbouring extremely type-specific neutralisation epitopes, where immunity to one HIV-1 variant via antibodies to PND is not sufficient for neutralisation even of a closely related virus strain [1,2]. In addition, the V3 region together with other parts of gp120 determines viral tropism for different permissive cell types [3–6]. Thus, cells of the monocyte/macrophage lineage and CD4-positive T-cells constitute two classes of target cells where the presence of one type of chemokine receptor, CCR5, determines macrophage tropism and another, CXCR4, determines T-cell tropism by aiding subsequent early interactions after the binding between HIV-1 and CD4 [7,8].

The different HIV-1 variants may not always appear to be strictly macrophage-tropic or strictly T-cell-tropic viruses, and several dual-tropic HIV-1 strains able to replicate in macrophages as well as in T-cells have been described [9–11]. Initially, it was described that dual-tropic viruses were able to bind both to CXCR4 of T-cells and to chemokine receptors of the CCR class of macrophages [11]. Recently it was described that also strictly CXCR4-binding HIV variants under certain conditions may be dual-tropic. Most cells of the monocyte/macrophage lineage carry CXCR4 molecules on the cell surface in addition to CCR5, normally acting as a secondary receptor for macrophage-tropic virus, but these CXCR4 molecules are not sufficient to promote infection by most HIV-1 variants only binding to CXCR4 [12]. For the new type of dual-tropic viruses this block is circumvented by an increased affinity for CXCR4, bypassing the normal restriction of replication of T-tropic HIV-1 [13]. The increased affinity for CXCR4 of such dual-tropic HIV-1 variants permits them to infect cells in a CD4-independent way.

We and others have shown that the *N*-linked glycan attached to N306 of the V3 loop plays a pivotal role in determining the accessibility of this region for neutralising antibodies. Thus, a mutant virus lacking this particular glycan is neutralised several hundred times more efficiently than is the wild type virus [14–16]. Recent data indicate that the shielding effect by the N306 glycan on the PND is restricted to protecting gp120 in oligomers from attack by anti-V3 antibodies, whereas monomeric gp120 is fully accessible even in the presence of the glycan [17]. This raises the question if the N306 also can modulate V3-dependent functions of gp120 such as cellular tropism. A recent paper by Nakayama et al. [18] suggested that elimination of this particular glycan would reduce the ability of T-tropic HIV to bind to CXCR4 and hence its ability to infect T-cell lines. These results appear to be at variance with both our data [16] and the data of Back et al. [14]. In the present paper, we extend our studies on the role of the N306 glycan for the biological properties of gp120. Specifically we asked the following questions: (i) Does the increased exposure of the V3 region induced by elimination of the N306 glycan expand the chemokine receptor use of strictly CXCR4-binding HIV to include also CCR5? (ii) If not, will the induced change in glycosylation induce dual-tropic behaviour due to an increased affinity for CXCR4? (iii) Finally we sought to evaluate if the loss of the glycan resulted in an opposite behaviour, as suggested by Nakayama et al. [18].

2. Materials and methods

2.1. HIV-1 clones

In most experiments we used an infectious clone of HIV-1, HIV-1_{A308}, lacking the glycosylation site at Asn306, generated by subject-

*Corresponding author.

PII: S0014-5793(99)00740-1

ing the proviral plasmid vector pBRU-2, containing the entire HIV-1_{BRU} genome, to site-directed mutagenesis [15,16]. The transfection and subsequent preparation of stock suspensions of mutant or wild type virus for further experiments were done under standardised conditions to ensure that no differences in spontaneous inactivation and degeneration of the two virus preparations occurred [19]. The mutation was confirmed by sequencing using the chain termination method, and the sequences were read in an ABI Prism automated capillary sequence reader (Applied Biosystems, Foster City, CA, USA) and processed using the Sequence Navigator Software (Applied Biosystems). The lack of one glycosylation site was further confirmed by SDS-PAGE of [^{3}H]GlcN-labelled mutant and wild type gp120 after immunoprecipitation (Fig. 1).

2.2. Cells

The CD4+ lymphocyte cell line H9 (ATCC HTB-176) [20,21] was cultured at 37°C and 5% CO_2, in RPMI 1640 supplemented with 20% heat-inactivated foetal calf serum (FCS), 2 μM glutamine, 100 IU of penicillin per ml, 20 μg gentamicin per ml and 100 IU streptomycin per ml. The CD4+ lymphocyte cell line MT-2 [22] was maintained in RPMI 1640 supplemented with 10% heat-inactivated FCS, 100 IU penicillin per ml, 20 μg gentamicin per ml and 100 IU streptomycin per ml. The monocytic cell line U937 (ATCC CRL 1593) was maintained in RPMI 1640 containing 10% heat-inactivated FCS, 2 μM glutamine, 100 IU of penicillin per ml, 20 μg gentamicin per ml and 100 IU streptomycin per ml. HOS-CD4 cells expressing CXCR4 (HOSCD4-CXCR4), CCR5 (HOSCD4-CCR5) and pBABE-puro (HOSCD4-pBABE) were obtained through the NIH AIDS Research and Reference Reagent Program and propagated as described by Deng et al. [23] and Laundau et al. [24]. HeLa-CD4 cells [25] were maintained in DMEM10 supplemented with 1 μg/ml G418 (Geneticin, Gibco) and 293 cells [26] were maintained in DMEM10. Peripheral blood mononuclear cells (PBMCs) were obtained from healthy blood donors. All cell cultures were maintained at 37°C in 5% CO_2.

2.3. Preparation of reporter virus by env trans-complementation

The 2.1 kb *Kpn*I-*Bam*HI fragment of the HIV-1 env trans-complementation vector pSVIIIenv [27] was exchanged with the corresponding fragments of pBRU-2 and pBRU-A308 env ORF resulting in the vectors pSV-BRU and pSV-BRU-A308, respectively, using general DNA subcloning techniques. An env-defective derivative of pSVIIIenv, pSV-dBgl, containing a 580 bp out of frame deletion within the env gene was used as a mock plasmid to determine background CAT activity. Virus capable of single-cycle replication was then generated by cotransfecting as indicated either COS-1 or 293 cells with env-expressing plasmid and pHXBΔenvCAT (an env-defective HIV expression vector containing a chloramphenicol acyltransferase (CAT) gene in the nef open reading frame of the HIV-1 genome [27]). COS-1 cells were transfected by lipofection using Lipofectin (Gibco) as previously described [28]; 293 cells were transfected by calcium phosphate precipitation exactly as described elsewhere [29]. Twenty-four hours after transfection culture medium was changed and after an additional 48 h virus was harvested and clarified by membrane filtration (45 μm). The virus particles thus generated were designated BRU and BRU-A308, respectively, to distinguish them from the corresponding fully infectious virus variants HIV-1BRU and HIV-1A308, also used in the present study.

2.4. Leu3a inhibition of A308 and BRU

A308 and BRU particles were produced by transfection of 293 cells as described above. Monoclonal antibody (mAb) Leu3a, specific for the gp120-binding domain of CD4 [30], in fivefold dilution was added to HeLa-CD4 [25] cultures in 24 well cluster dishes, containing 400 μl of Dulbecco's modification of Eagle's MEM. Media from BRU- or A308-producing 293 cells were passed through a 45 μm membrane filter and 400 μl of BRU or A309 suspension was added to the Leu3a-containing HeLa-CD4 wells. The cultures were incubated at 37°C for 3 days. Subsequently, the HeLa-CD4 cells were lysed and assayed for CAT activity as previously described [28].

2.5. Single-cycle infectivity assay

Single-cycle replication conditions was ascertained by env trans-complementation by pSV-BRU and pSV-BRU-A308 in COS-1 cells as described by Helseth et al. [27] and as modified by Lund et al. [28]. HIV-1 infection of the target cell line, MT4, was normalised to transfection efficiency of HIV-1 producer cells using CAT activity of COS-1 cells as the normalisation factor. COS-1 cells were lysed in 400 μl Reporter lysis buffer (Promega) after harvest of reporter virus supernatant.

2.6. Antigen production kinetics assay

H9 cells were transfected with pBRU-2 or pBRU-A308 by DEAE-dextran [0.3×10^6 cells/ml in RPMI 1640 (Gibco); DNA 2 μg/ml; DEAE dextran (MW 500 000; Pharmacia Upjohn, Stockholm, Sweden) 7 μg/ml; incubation at 37°C for 45 min]. Cells were then washed, and seeded on 24 well tissue culture plates (Nunclon, Roskilde, Denmark) in RPMI 1640 containing 10% FCS and viral antigen was allowed to peak (3 weeks culture). Then uninfected H9 cells were mixed with 1/500 cells from infected cultures and seeded on 6 well tissue culture plates (Nunclon) in 2.5 ml at 0.1×10^{-6} cells/ml. Samples of supernatant were harvested daily (day 5 to day 11) and cultures were diluted 1:5 on day 4 and day 7. HIV-1 antigen was quantified by an in house ELISA based on polyclonal antibodies obtained from HIV-1 infected patients [31]. Frozen stock dilutions of supernatants from H9 cells chronically infected with HIV-1_{LAI} were used as internal standards [32].

2.7. Infectivity assay

Mutant and wild type virus variants were produced and titrated in H9 cells according to the principle of Reed and Muench to determine $TCID_{50}$ of each virus [33]. Subsequently, 1600 $TCID_{50}$ of each virus variant were inoculated to 7.5×10^5 U937 cells in quadruplicate in 24 well cell culture plates (1.5 ml medium per well) and titrated at fivefold dilutions. The same virus variants were also analysed in MT4, H9 and PBMC cultures, using 100 $TCID_{50}$ per culture well. The infected cultures were incubated at 37°C, and virus antigen (p24) in culture supernatant was measured at various times post infection, as described by Valhne et al. [34]. Similar conditions were used for infection of PBMC.

2.8. Syncytium formation assay

The extent of syncytium formation by the mutant and wild type virus variants was determined mainly according to Watkins et al. [35]. H9 cells were infected with HIV-1_{BRU} or HIV-1_{A308} as described above. After the infected cell cultures had passed the syncytium formation phase, but were still expressing high levels of p24 in culture supernatant, infected cells were washed once in culture medium and mixed with uninfected H9 cells (ratio of infected:uninfected cells 1:5). The number of syncytia in cell cultures was determined 48 h after mixing infected and uninfected cells. mAb NEA-9205 (1.5 μg/ml) and a pool of sera from ARC patients (corresponding to 25 μl serum) were used in blocking experiments (adsorption at 37°C for 1 h) to control the specificity of syncytia formation.

3. Results

First we investigated if the increased exposure of the V3 region due to loss of the N306 glycan resulted in expanded co-receptor usage of a prototype T-tropic virus. Chimeric reporter virus BRU-A308, lacking the glycosylation site at N306, and corresponding wild type reporter virus BRU, both expressing CAT activity when taken up by permissive cells, were produced by trans-complementation as described by Lund et al. [28]. It was found that both BRU and BRU-A308 produced CAT activity after infection of CD4-CXCR4-expressing HOS cells whereas corresponding CCR5-expressing cells were refractive to BRU as well as BRU-A308 (Table 1). The infection of HOSCD4-CXR4 by BRU and BRU-A308 was blocked equally well by the anti-CD4 antibody Leu3a, indicating that both mutant and wild type virus were dependent on CD4 for infection of permissive cells (Fig. 2a). Altogether, these data demonstrated that increased exposure of the V3 loop in a T-tropic HIV strain does not confer CCR5-dependent tropism on such viruses. Moreover, our data did not suggest that the CXCR4-dependent entry of the carbohydrate-

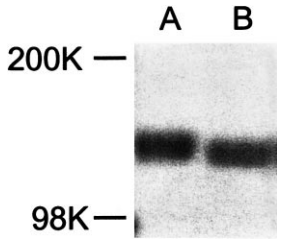

Fig. 1. RIPA and SDS-PAGE of immunoprecipitated gp120 from culture media of H9 cells infected with HIV-1_{BRU} (lane A) or HIV-1_{A308} (lane B). The positions of molecular weights are indicated in the figure.

deficient mutant was impaired compared to wild type virus as has been suggested by Nakayama et al. [18].

The increased uptake rate of BRU-A308 in CXCR4-expressing HOS cells could possibly reflect a higher fusion capacity of mutant virus compared with wild type virus. Next, we therefore investigated if the *N*-linked glycan of N306 interfered with the ability of virus-infected cells to form syncytia according to the method of Watkins et al. [35] (Table 2). These results demonstrated that HIV-1_{A308} did not induce syncytium formation significantly better than did HIV-1_{BRU}, suggesting that the N306 glycan is not responsible for interference with post-binding interactions necessary for syncytium formation. However, mAb NEA-9205, binding to a V3 epitope, blocked the syncytium formation capacity of HIV-1_{A308} to a higher extent than that of HIV-1_{BRU}, which was not unexpected owing to the possible involvement of the V3 region in HIV-induced fusion events [36].

These results did thus not exclude the possibility that elimination of the N308 glycan could facilitate HIV-1 infection of normally low-permissive CXCR4-positive cells in other ways, resulting in an expansion of the range of permissive cells. To determine if the elimination of the N306 glycosylation site resulted in a switch to dual tropism via the alternative mechanism, i.e. by enhanced binding to CXCR4, we infected a clone of the monocytoid cell line U937, normally refractory to T-tropic HIV [12] variants, with HIV-1_{A308} or HIV-1_{BRU}.

Table 1
Co-receptor usage of HIV-1_{A308}

HIV construct[a]	Target cell	CAT signal (cpm)
BRU	HOS-CD4-CCR5	719
		167
A308	HOS-CD4-CCR5	1 003
		523
BRU	HOS-CD4-Fusin	17 430
		18 380
A308	HOS-CD4-Fusin	32 849
		39 109
BRU	HOS-CD4-pBABE	224
		285
A308	HOS-CD4-pBABE	877
		803
BRU	HeLa-CD4	18 686
		16 788
A308	HeLa-CD4	30 214
		29 227

[a]Carried out essentially as described by Lund et al. [28].

As a positive control, we used HIV-1_{IIIB}, clone strain New York, readily infecting U937 cells (Bolmstedt et al., unpublished). Although a high dose of virus (500 $TCID_{50}$ per culture) was used, we found that neither HIV-1_{A308}- nor HIV-1_{BRU}-infected U937 cells produced any detectable amounts of p24 antigen. In contrast, a marked increase in p24 antigen production in B24-infected cells was observed on day 14 (Fig. 2b). These data show that elimination of the N306 glycan is not sufficient to introduce tropism for monocytoid cell lines such as U937 in a CXCR4-dependent manner.

To determine whether the phenomenon described by Nakayama et al. [18], i.e. that elimination of the N306 glycan should result in a decrease in the ability of HIV-1 to replicate in normal immortalised T-cell lines, we analysed the kinetics of mutant and wild type virus infection in several ways. In the first series of experiments, we used BRU and BRU-A308 in a single-cycle HIV-1 replication assay, using the trans-complementation system of Helseth et al. [27]. The envelope-defective HIV-1 reporter vector, pHXBΔenvCAT, was cotransfected with the envelope expression vectors pSV-BRU and pSV-

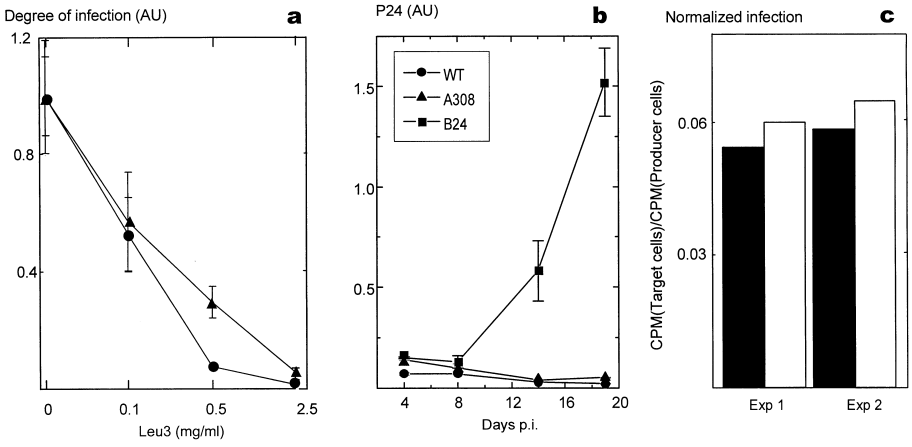

Fig. 2. a: Leu3a neutralisation-dependent neutralisation of HIV-1_{BRU} (circles) and of HIV-1_{A308} (triangles). b: Lack of replication of HIV-1_{A308} and HIV-1_{BRU} in monocytoid U937 cells. U937 cells were infected with 1600 $TCID_{50}$ of HIV-1_{IIIB} (strain B24), HIV-1_{A308}, or HIV-1_{BRU}. The concentration of p24 antigen (arbitrary units) was measured at different times as indicated in the figure. c: Single-cycle replication of HIV-1. HIV-1 env trans-complementation was performed using the envelope expression vectors pSV-BRU and pSV-BRU-A308, derived from pSVIIIenv [27]. CAT activity of target cells, MT4, was normalised to CAT activity determined in the transfected producer cells, COS-1, in order to correct for differences in transfection efficiency. Results of two separate experiments are shown.

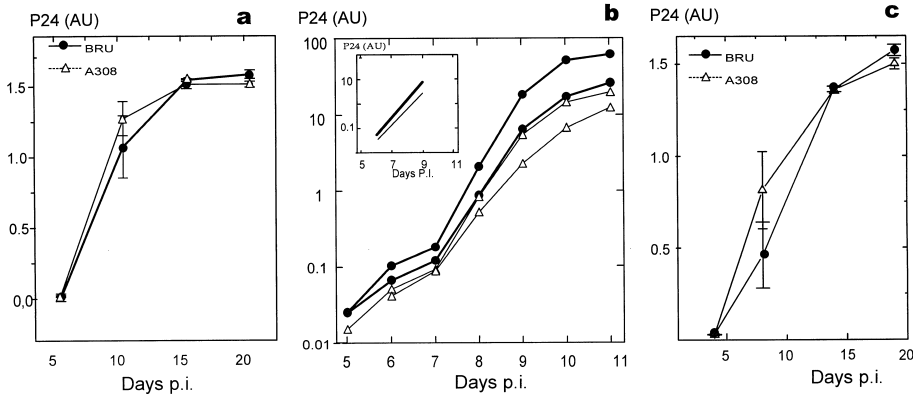

Fig. 3. a: Long-term replication of HIV-1_{A308} and HIV-1_{BRU} in MT2 cells. About 100 $TCID_{50}$ HIV-1_{A308} or HIV-1_{BRU} was added to each culture well. The concentration of p24 antigen (arbitrary units) was measured at different times as indicated in the figure. b: HIV-1 antigen production analysis in H9 cultures inoculated at day 0 with 1/500 cells infected with either BRU (bold line; solid circles) or BRU-A308 (thin line; open triangles). Two parallel inoculations were performed for each viral isolate. Antigen is determined as arbitrary units (AU) and depicted logarithmically. Cell cultures were diluted 1:5 on day 4 and day 7 and the measured HIV-1 antigen was subsequently multiplied with the accumulated dilution factor (5× day 5–7; 25× day 8–11). Inset: Linear regression of the data for BRU (solid line) and BRU-A308 (thin line). c: Long-term replication of HIV-1_{A308}, and HIV-1_{BRU} in H9 cells. About 100 $TCID_{50}$ HIV-1_{A308} or HIV-1_{BRU} was added to each culture well. The concentration of p24 antigen (arbitrary units) was measured at different times as indicated in the figure.

BRU-A308 into COS-1 cells by lipofection. After 48 h the resultant HIV-1 supernatant was transferred to MT4 cells, and the resultant infection (determined as CAT activity) was determined and normalised to transfection efficiency of the COS-1 cells. The CAT activity determined in COS-1 cells following harvest of HIV-1 supernatant was used as the normalisation factor. We found that infectivity of BRU and BRU-A308 envelope in MT4 cells was almost identical by this assay (Fig. 2c).

A similar result was obtained also when replication of HIV-1_{A308} and HIV-1_{BRU} was compared in MT2 cells over 20 days (Fig. 3a). Thus, both HIV-1_{BRU} and HIV-1_{A308} displayed essentially congruent growth curves, reaching a maximum of produced p24 on day 15. Both these results demonstrated that the induced mutation in HIV-1_{A308} did not change the ability of HIV-1 to replicate in HTLV-1 transformed cells such as MT4.

We also performed a comparison in H9 cells of HIV-1 antigen kinetics between BRU and A308. Uninfected H9 cells were inoculated with 500 times less H9 cells, transfected with pBRU2 and or pBRUA308, and cultured for 11 days (Fig. 3b). Linear regression of the logarithmic plot of HIV-1 antigen (day 6 to day 9) showed no gross difference between the calculated linear coefficients of BRU (0.73 ± 2) and BRU-A308 (0.65 ± 0.16) [95% confidence limits indicated]. Finally we determined whether the long-term replication kinetics in H9 (Fig. 3c) and diploid PBMC (Fig. 4) was affected by elimination of the N306 glycan. No significant difference in the replication kinetics was observed between mutant virus and wild type virus in these experiments.

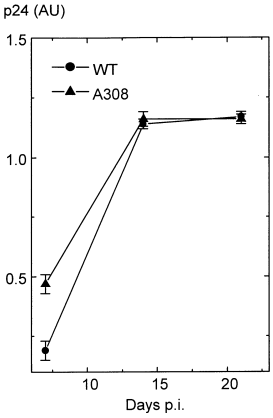

Fig. 4. Long-term replication of HIV-1_{BRU} and HIV-1_{A308} in PBMC. About 100 $TCID_{50}$ HIV-1_{A308} or HIV-1_{BRU} was added to each culture well. The concentration of p24 antigen (arbitrary units) was measured at different times as indicated in the figure.

4. Discussion

We and others have demonstrated a key role of the N306 *N*-linked glycan in shielding the tip of the V3 region from neutralising monoclonal and polyclonal antibodies [14,15,29]. Owing to the critical role of this gp120 region, it is reasonable that elimination of this glycan would result in a virus with altered growth properties. Although there is evidence that elimination of other *N*-glycosylation sites in gp120 could change the three-dimensional conformation of the glycoprotein [37], all available data suggest that elimination of the glycosylation site at A308 results in increased exposure of the V3 region of gp120 oligomers, but without any detectable change in the antigenic conformation. This conclusion stems from the observations that several polyclonal and monoclonal antibodies to the V3 region, prepared after immunisation with monomeric wild type gp120, bound several times better to mutant gp120 oligomers than to corresponding

Table 2
Syncytium induction of HIV-1_{BRU} and HIV-1_{A308} in H9 cells

Virus/cell	Medium	NEA-9205	ARC serum
HIV-1_{BRU}/H9	48	17	3
	45	19	2
HIV-1_{A309}/H9	45	7	0
	44	5	0
H9	0	0	0
	0	0	0

Data shown as number of syncytia per well ($n = 2$) of infected H9 cells 48 h post infection.

wild type aggregates. In contrast, wild type and mutant monomeric gp120 bound equally well to antibodies to V3 [17,29]. Therefore, the expected change, if any, as to replication efficiency introduced by elimination of the *N*-glycosylation site at A308 would be an increased growth rate as a consequence of increased exposure of gp120 domains involved in the early interactions between HIV-1 and its host cell.

With this background, the result of Nakayama et al. [18] is surprising as they suggest a significant impairment of replication efficiency of mutant virus, lacking the N306 glycan. One explanation suggested by Nakayama et al. [18] for the discrepancy between their results and the results obtained in our laboratory [15,16] and by Back et al. [14] is that the difference is subtle and not observable in short-term replication experiments. However, in the present paper, we extended the observation period to saturation at 20 days post infection using the same multiplicity of infection as Nakayama et al. [18] without observing any detectable difference between mutant and wild type virus. Moreover, the experiments performed on the single-round replication did not reveal any difference in replication rate between mutant and wild type virus.

One possible explanation for the discrepancy between the results of the present paper and those of Nakayama et al. [18] is that these authors introduced relatively large amino acid changes, when eliminating the *N*-glycosylation site at N306. Thus, for one mutant the uncharged polar N306 was replaced by a positively charged His and for other mutants uncharged polar Thr308 was changed to an isoleucine with a large hydrophobic side chain, which should be compared with our strategy where Thr306 was changed to Ala, with a small hydrophobic side chain only. In other words, the effects observed by Nakayama et al. [18] could be caused by the prominent chemical difference introduced in the amino acid side chains without any involvement of the *N*-linked glycan. In fact, we recently showed that replacing a Thr of a glycosylation site of the herpes simplex virus glycoprotein C by an isoleucine resulted in a prominent change in its binding behaviour without involving the glycan itself [38]. It should be pointed out that the wild type virus used in our study had the same amino acid sequence over the entire V3 region as strain NL41, used in the study by Nakayama et al. [18].

This would also suggest that the second phenomenon observed by Nakayama et al., i.e. that clinical HIV-1 isolates lacking the N308 glycan often contain an additional amino acid substitution in the tip of the V3 region, is of passenger nature and not a compensation for decreased CXCR4 binding suggested by these authors. Rather, this could reflect our observation [17] that mutant virus lacking the N308 glycan became two orders of magnitude more susceptible than corresponding wild type virus to neutralisation by anti-V3 antibodies and that escape mutants with regained resistance frequently occurred where amino acids within or close to the V3 region were changed to basic ones similar to the findings of Nakayama et al. [18].

The present data demonstrated that the increased exposure of the V3 region in intact virus particles did not enhance the infectivity of mutant HIV-1_{A308} in immortalised cell lines nor did it alter the fusion capacity in terms of syncytium formation. It is, however, noteworthy that the syncytium formation activity in HIV-1_{A308}-infected cells was significantly more susceptible to neutralisation by anti-V3 antibodies compared with correspondingly HIV-1_{BRU}-infected cells. This result suggests that the N306 glycan sterically affects the gp41/gp120 domains responsible for fusion at least to some extent. However, the fact that the general infectivity neither in short-term nor in long-term propagation is affected by the mutation suggests that the fusion function such as observed in syncytium formation is not a rate-limiting step in the viral infection of permissive immortalised or diploid target cells. Altogether, our results suggest that the conclusion of Nakayama et al. [18], that the V3 glycan of HIV-1 in analogy with corresponding V3 glycan of SIV_{mac} [39] constitutes a strong determinant for host cell range, may not be universal.

Acknowledgements: We thank Richard Lymer, Barbro Wiksten, and Annika Hallgren for skilful technical assistance. The following reagents were obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH: HOS-CD4-CCR5, HOS-CD4-Fusin, HOS-CD4-pBABE-puro from Dr. Nathaniel Landau, HeLa-CD4 clone 1022 from Dr. Bruce Chesebro, and 293 from Dr. Andrew Rice. This work was supported by Grants from the Swedish Medical Research Council (9083 and 10437) and the Medical Faculty, University of Göteborg.

References

[1] Arendrup, M., Sonnerborg, A., Svennerholm, B., Akerblom, L., Nielsen, C., Clausen, H., Olofsson, S., Nielsen, J.O. and Hansen, J.E. (1993) J. Gen. Virol. 74, 855–863.
[2] Palker, T.J., Clark, M.E., Langlois, A.J., Matthews, T.J., Weinhold, K.J., Randall, R.R., Bolognesi, D.P. and Haynes, B.F. (1988) Proc. Natl. Acad. Sci. USA 85, 1932–1936.
[3] Chesebro, B., Wehrly, K., Nishio, J. and Perryman, S. (1992) J. Virol. 66, 6547–6554.
[4] Chesebro, B., Wehrly, K., Nishio, J. and Perryman, S. (1996) J. Virol. 70, 9055–9059.
[5] Hwang, S.S., Boyle, T.J., Lyerly, H.K. and Cullen, B.R. (1992) Science 257, 535–537.
[6] Liu, Z.Q., Wood, C., Levy, J.A. and Cheng Mayer, C. (1990) J. Virol. 64, 6148–6153.
[7] Bleul, C.C., Farzan, M., Choe, H., Parolin, C., Clark-Lewis, I., Sodroski, J. and Springer, T.A. (1996) Nature 382, 829–833.
[8] Oberlin, E., Amara, A., Bachelerie, F., Bessia, C., Virelizier, J.L., Arenzana-Seisdedos, F., Schwartz, O., Heard, J.M., Clark-Lewis, I., Legler, D.F., Loetscher, M., Baggiolini, M. and Moser, B. (1996) Nature 382, 833–835.
[9] Cho, M.W., Lee, M.K., Carney, M.C., Berson, J.F., Doms, R.W. and Martin, M.A. (1998) J. Virol. 72, 2509–2515.
[10] Trkola, A., Ketas, T., Kewalramani, V.N., Endorf, F., Binley, J.M., Katinger, H., Robinson, J., Littman, D.R. and Moore, J.P. (1998) J. Virol. 72, 1876–1885.
[11] Doranz, B.J., Rucker, J., Yi, Y., Smyth, R.J., Samson, M., Peiper, S.C., Parmentier, M., Collman, R.G. and Doms, R.W. (1996) Cell 85, 1149–1158.
[12] Moriuchi, H., Moriuchi, M., Arthos, J., Hoxie, J. and Fauci, A.S. (1997) J. Virol. 71, 9664–9671.
[13] Yi, Y., Rana, S., Turner, J.D., Gaddis, N. and Collman, R.G. (1998) J. Virol. 72, 772–777.
[14] Back, N.K., Smit, L., De Jong, J.J., Keulen, W., Schutten, M., Goudsmit, J. and Tersmette, M. (1994) Virology 199, 431–438.
[15] Hansen, J.-E.S., Jansson, B., Gram, G., Clausen, H., Nielsen, J.O. and Olofsson, S. (1996) Arch. Virol. 141, 291–300.
[16] Schønning, K., Jansson, B., Olofsson, S. and Hansen, J.-E.S. (1996) J. Gen. Virol. 77, 753–758.
[17] Schønning, K., Jansson, B., Olofsson, S., Nielsen, J.O. and Hansen, J.-E.S. (1996) Virology 218, 134–140.
[18] Nakayama, E.E., Shioda, T., Tatsumi, M., Xin, X.M., Yu, D.S., Ohgimoto, S., Kato, A., Sakai, Y., Ohnishi, Y. and Nagai, Y. (1998) FEBS Lett. 426, 367–372.
[19] Layne, S.P., Merges, M.J., Dembo, M., Spouge, J.L., Conley, S.R., Moore, J.P., Raina, J.L., Renz, H., Gelderblom, H.R. and Nara, P.L. (1992) Virology 189, 695–714.
[20] Gootenberg, J.E., Ruscetti, F.W., Mier, J.W., Gazdar, A. and Gallo, R.C. (1981) J. Exp. Med. 154, 1403–1418.

[21] Mann, D.L., O'Brien, S.J., Gilbert, D.A., Reid, Y., Popovic, M., Read-Connole, E., Gallo, R.C. and Gazdar, A.F. (1989) AIDS Res. Hum. Retroviruses 5, 253–255.
[22] Haertle, T., Carrera, C.J., Wasson, D.B., Sowers, L.C., Richman, D.D. and Carson, D.A. (1988) J. Biol. Chem. 263, 5870–5875.
[23] Deng, H., Liu, R., Ellmeier, W., Choe, S., Unutmaz, D., Burkhart, M., Di Marzio, P., Marmon, S., Sutton, R.E., Hill, C.M., Davis, C.B., Peiper, S.C., Schall, T.J., Littman, D.R. and Landau, N.R. (1996) Nature 381, 661–666.
[24] Landau, N.R. and Littman, D.R. (1992) J. Virol. 66, 5110–5113.
[25] Chesebro, B., Wehrly, K., Metcalf, J. and Griffin, D.E. (1991) J. Infect. Dis. 163, 64–70.
[26] Graham, F.L., Smiley, J., Russell, W.C. and Nairn, R. (1977) J. Gen. Virol. 36, 59–74.
[27] Helseth, E., Kowalski, M., Gabuzda, D., Olshevsky, U., Haseltine, W. and Sodroski, J. (1990) J. Virol. 64, 2416–2420.
[28] Lund, O.S., Losman, B., Schønning, K., Bolmstedt, A., Olofsson, S. and Hansen, J.-E. (1998) AIDS Res. Hum. Retroviruses 14, 1445–1450.
[29] Schønning, K., Bolmstedt, A., Novotny, J., Losman, B., Lund, O.S., Nielsen, J.O., Olofsson, S. and Hansen, J.-E.S. (1998) AIDS Res. Hum. Retroviruses 14, 1451–1456.
[30] Hansen, J., Nielsen, C., Clausen, H., Mathisen, L. and Nielsen, J. (1991) Antiviral Res. 16, 233–242.
[31] Hansen, J.-E.S., Nielsen, C., Arendrup, M., Olofsson, S., Mathiesen, L.R., Nielsen, J.O. and Clausen, H. (1991) J. Virol. 65, 6461–6467.
[32] Popovic, M., Sarngadharan, M., Read, E. and and Gallo, R. (1984) Science 224, 497–500.
[33] Reed, L.J. and Muench, H. (1938) Am. J. Hyg. 27, 493–497.
[34] Vahlne, A., Horal, P., Eriksson, K., Jeansson, S., Rymo, L., Hedstrom, K.G., Czerkinsky, C., Holmgren, J. and Svennerholm, B. (1991) Proc. Natl. Acad. Sci. USA 88, 10744–10748.
[35] Watkins, B.A., Crowley, R., Davis, A.E., Louie, A.T. and Reitz Jr., M.S. (1997) J. Gen. Virol. 78, 2513–2522.
[36] Page, K.A., Stearns, S.M. and Littman, D.R. (1992) J. Virol. 66, 524–533.
[37] Bolmstedt, A., Sjölander, S., Åkerblom, L., Hemming, A., Hansen, J.-E.S., Hu, S.-L., Morein, B. and Olofsson, S. (1996) J. Acquir. Immune Defic. Syndr. 12, 213–220.
[38] Olofsson, S., Biller, M., Bolmstedt, A., Mårdberg, K., Leckner, J., Malmstr öm, B.G., Trybala, E. and Bergström, T. (1999) Glycobiology 9, 73–81.
[39] Ohgimoto, S., Shioda, T., Mori, K., Nakayama, E.E., Hu, H. and Nagai, Y. (1998) J. Virol. 72, 8365–8370.