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Effects of mutations on HIV-1 infectivity and neutralization involving the conserved NNNT amino acid sequence in the gp120 V3 loop

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ABSTRACT

The N-glycan g15 within the HIV-1 gp120 V3 loop efficiently blocks antibodies to facilitate viral escape from humoral immune responses. However, we have isolated primary viruses all lacking the N-glycosylation site g15 due to mutations (NNNT > YRNA, HNTV, SIQK), which showed resistance to neutralizing antibodies present in autologous or heterologous HIV-1 positive sera. When introduced into the NL4-3 background, the sequences YRNA, HNTV and SIQK caused an increase of viral infectivity and resistance to neutralization. Thus, despite the lack of g15, primary isolates can escape from neutralization because of specific mutations of the NNNT sequence altering coreceptor usage.

1. Introduction

The external human immunodeficiency virus type-1 (HIV-1) envelope glycoprotein gp120 is a highly glycosylated protein with more than 60% of its molecular mass being carbohydrate structures [1]. These complex carbohydrates, especially within the gp120 V3 loop, are able to block binding of neutralizing antibodies [2–4] to allow escape from humoral immune responses [5]. An efficient viral entry into target cells requires the interaction of the gp120 with (i) heparansulfateproteoglycan [6], (ii) the CD4 receptor [7] and (iii) at least one of the two coreceptors CXCR4 or CCR5 [8]. A region of gp120 that binds to the coreceptors is the hypervariable V3 loop [9,10]. Within the V3 loop sequences of subtype B viruses, a conserved sequence motif $N^{81}N^{98}N^{96}T^{97}$ [11] is present, which contains the N-glycosylation site g15 (counting for NL4-3). By sequence data analysis it was revealed that NNT is present in 96% of all B subtype V3 loop sequences listed [11]. Mutations within the NNT motif can destroy the original g15 N-glycosylation site but the additional N amino acid of the NNNT motif can rescue the N-glycosylation site [12]. All these data demonstrate that the g15 N-glycosylation site within the V3 loop is highly conserved. This further suggests that in vivo a strong selective pressure for the presence of the g15 N-glycan might exist.

An important function of N-glycan g15 is the shielding of the V3 loop [13], leading to the highly efficient blockade of neutralizing antibody [2–5,14–16]. Another role of g15 is its supportive role for the infection of cells when CCR5 is used as a coreceptor [17]. R5-tropic viruses showed higher infection rates when g15 was present and infection was impaired by mutations destroying the NNT g15 site [4]. For CXCR4-specific infection the function of N-glycan g15 is completely the opposite [4,13]. X4-tropic viruses lacking g15 because of a single amino acid exchange N301Q, showed higher infection rates but these viruses become highly sensitive to neutralization [13,18,19]. From all these experiments it was concluded that N-glycan g15 is blocking neutralizing antibody and viruses lacking g15 become highly sensitive to neutralization by V3-loop specific antibodies.

In general, the exchange NNT > QNT is a well-accepted method to transform a functional N-glycosylation site into a non-functional site to study the role of complex carbohydrates in HIV-1 infection [4,13,20,21], but the QNT sequence is not detectable in patient derived HIV-1 primary isolates. In our study, we now have identified primary viruses lacking the N-glycosylation site for N-glycan g15. These primary viruses showed an atypically mutated g15 region where 3–4 of 4 amino acids were replaced (NNNT amino acids, 300–303, Fig 1A). However, such atypical mutations have also been identified in other primary isolates [22] or are present in V3 sequences published by the HIV sequence database [11]. As mentioned before, mutations within the V3 loop affect the usage of coreceptors, antibody binding and N-glycosylation. Since the V3

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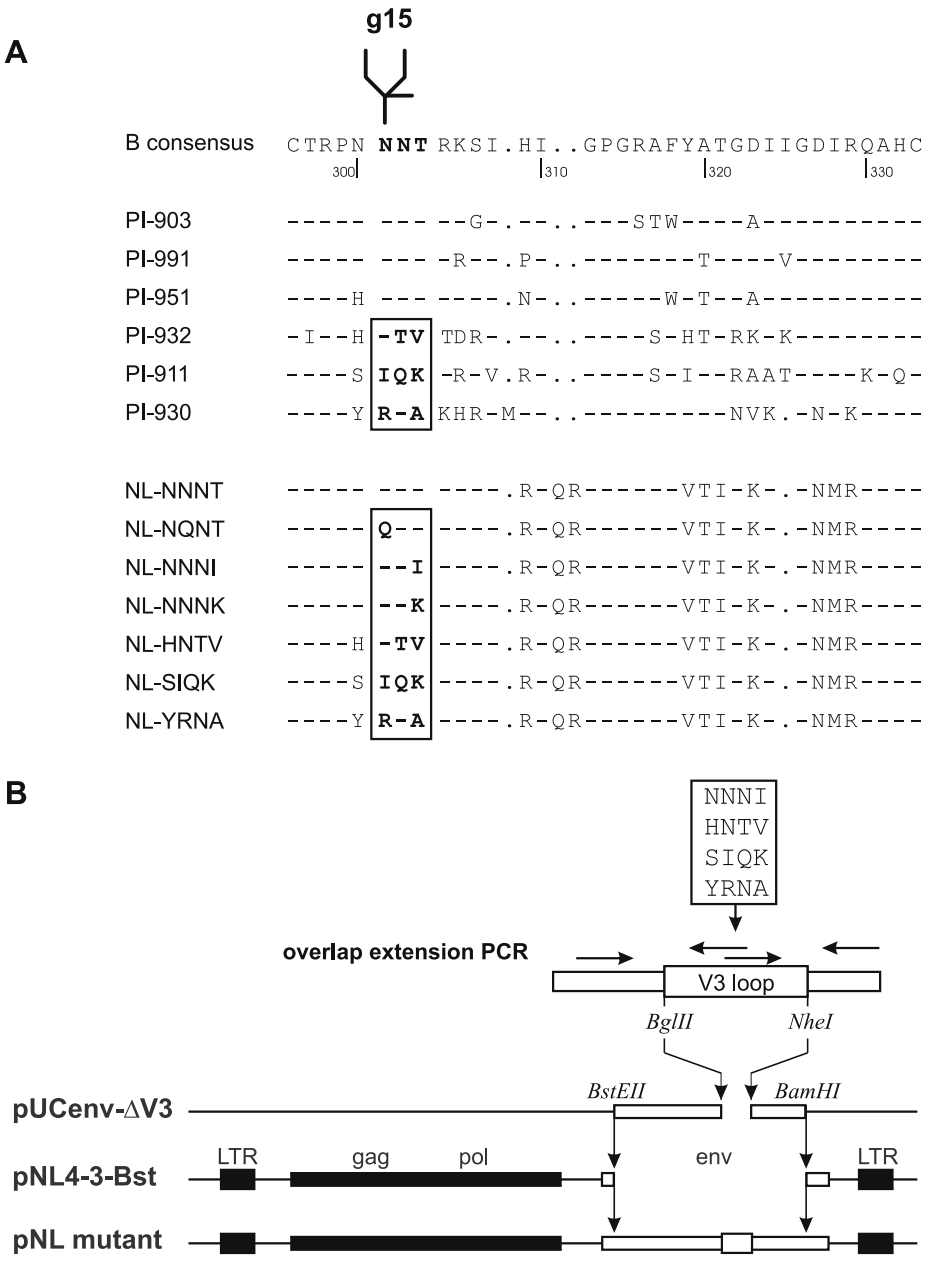

Fig. 1. V3 loop sequences of primary isolates and construction of NL4-3 mutants. (A) V3 loop sequences were aligned to the B clade consensus sequence. Boxed, mutated N-glycosylation sites. PI, primary isolates. NL, these NL4-3 mutants are differing in amino acids position 300–303. (B) Construction of pNL mutants. Proviral DNA from primary isolate infected cells was PCR amplified and cloned into bacterial plasmid pCR™. A single characterized bacterial clone was used for mutagenesis by overlap extension PCR to generate the V3 loops only differing in the NNNT amino acid sequnece. The V3 PCR fragment was cloned into pUCenv-ΔV3 and characterized by DNA sequencing. From the pUCenv plasmid the env gene was cloned as a BstEII-BamHI fragment into the pNL4-3-Bst vector to generate a functional provirus.

loop is an important determinant of viral infection, changes of the V3 loop amino acid sequence will have a significant impact on coreceptor usage and the efficiency of viral entry. The efficiency of viral entry can be estimated as the specific infectivity of a virus mutant to allow comparison between different viruses.

Here, we have investigated the role of mutated NNNT motifs (YRNA, SIQK, HNTV) from V3 loops of primary isolates for viral infectivity and neutralization in the context of primary viruses and in the context of mutated NL4-3 viruses. Our data suggest that the lack of protection, provided by the N-glycan g15 against neutralizing antibodies, can be compensated by specific amino acid mutations in the NNNT region, which enhanced the specific infectivity of the respective virus mutant.

2. Materials and methods

2.1. HIV-1 primary isolates

The six primary isolates were obtained from serum samples of five AIDS patients. All AIDS patients were anti-HIV-1 seropositive as confirmed by standard Western immunoblot and enzyme immunoassay and were classified as stages III–IV c–d according to the Centers for Disease Control (CDC) classification. To isolate the primary virus, PBMC-free serum samples were applied to phytohemagglutinin (PHA) and interleukin-2 (IL-2) activated HIV-1 negative donor PBMC as described before [22]. Briefly, donor PBMC