AIDS RESEARCH AND HUMAN RETROVIRUSES
Volume 12, Number 16, 1996
Mary Ann Liebert, Inc.

Short Communication

V3 Loop Sequence Analysis of Seven HIV Type 1 Group O Isolates Phenotyped in Peripheral Blood Mononuclear Cells and MT-2 Cells

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ABSTRACT

HIV-1-infected individuals from which syncytium-inducing (SI) viruses are isolated most often progress more rapidly to AIDS than individuals carrying only non-syncytium-inducing (NSI) viruses. The syncytium-inducing capacity of virus isolates is commonly determined in conjunction to replication in MT-2 cells. Comparison of HIV-1 *env* sequences and a site-directed mutagenesis study have indicated that the presence of a positively charged amino acid at position 11 or 25 in the V3 loop is minimally required for the SI capacity of HIV-1 subtype B viruses. Studies have also shown a similar correlation between positively charged signature amino acids in the V3 loop and syncytium formation in MT-2 cells for HIV-1 subtypes A, D, and E. In the present study virus phenotype was determined and compared to the V3 loop sequence of seven HIV-1 group O isolates. Three of the HIV-1 group O isolates showed the NSI/non-MT-2 tropic phenotype and two showed the SI/MT-2 tropic phenotype, whereas two isolates presented an uncommon NSI/MT-2 tropic phenotype. The V3 loop of the two SI/MT-2 tropic isolates had a high net positive charge and contained a positively charged amino acid at position 11 or 25. The V3 loop of the two NSI/MT-2 tropic isolates had a low net positive charge and contained a single positively charged amino acid at position 37.

HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 subtype B isolates can be divided into two groups on the basis of their syncytium-inducing capacity in peripheral blood mononuclear cells (PBMCs).[1–4] In contrast to non-syncytium-inducing (NSI) viruses, syncytium-inducing (SI) isolates are characterized by their ability to establish continuous replication and induce the formation of syncytia in MT-2 cells.[5,6] The emergence of SI isolates in the course of infection heralds rapid $CD4^+$ cell decline and disease progression.[7] Studies involving site-directed mutagenesis within the V3 loop[8–11] and exchange of the V3 loop of HIV-1_{HXB-2} with naturally occurring V3 loops of NSI and SI viruses have shown that determinants of the SI phenotype are located within the V3 loop.[12] Subsequently, an association was demonstrated between the presence of a positively charged amino acid at positions 11 and 25 in the V3 loop and syncytium-inducing capacity.[13] The role of positively charged amino acids at these positions in conferring the SI phenotype was formally documented by site-directed mutagenesis.[14] These signature amino acids have also been shown to play a key role in determining the syncytium-inducing capacity of HIV-1 subtypes A, D, and E viruses.[15,16] In these studies, the SI phenotype of HIV-1 subtypes was assessed by determining MT-2 tropism. In contrast, a lack of correlation between the SI phenotype and positively charged amino acids at positions 11 and 25 has been described for HIV-1 subtype F isolates from Romania.[17] Further investigation has indicated that while the V3 loop of

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subtype F isolates from Romania clearly harbors determinants of MT-2 tropism, the formation of syncytia occurs both in the presence and absence of positively charged signature amino acids (C. Holm-Hansen and J.J. de Jong, in preparation).

A new group of HIV isolates has been obtained that is phylogenetically more closely related to HIV-1 than to HIV-2.[18–20] These isolates have, however, only modest homology with HIV-1 group M isolates and have therefore been placed in a separate "outlier" group referred to as HIV-1 group O.[21,22] HIV-1 group O viruses are primarily found in Cameroon and Gabon and have only recently spread to Europe.[21,23] While HIV-1 group O viruses cause AIDS, little is known about the virulence of these viruses. Because virus phenotype has been associated with the course of HIV-1 infection it was of interest to assess the presence of different phenotypes of group O viruses collected at various stages of HIV infection. In addition, an attempt was made to map phenotypic determinants to specific positions in the V3 loop.

Seven primary isolates of HIV-1 group O viruses were included in the present study. Two of these isolates were obtained sequentially from patient ANT70 and two were sequential isolates from her partner, ANT70-NA.[18,20] The remaining isolates, BCF02, BCF06, and BCF07, were obtained from three Cameroonian individuals living in France and reported to be infected with group O viruses.[24] To determine the relationship between these seven virus isolates and the previously characterized HIV-1 group O isolates, we analyzed part of the envelope gene including the V3 loop of viruses obtained from PBMC culture supernatants. RNA was isolated from cell-free PBMC supernatants, reverse transcribed into cDNA, and amplified by

TABLE 1. DERIVED AMINO ACID SEQUENCE OF THE SEVEN PRIMARY ISOLATES[a]

Isolate	*Sequence*			
		<—— V3	11	——
Consensus O	LSKGKIR?MGK.NISD?GKNIIVTLNSTI?MT	CERPGN?TVQ	E	I?IGPMA
ANT70.1	-------M-A-.---GS-E---------LN--	-K--AM.K--	-	MR-----
ANT70.2	-------M-A-.---GS-E---------LN--	-K--AM.K--	-	MR-----
ANT70-NA.1	---E---M-AR.---NN-R---------LNI-	-R--EI.KR-	-	VK----S
BCF02	--E----M-A-.----S-Q-------T--N--	-Q---HQ---	-	-R-----
BCF07	-------L-A-.----S-Q-------T--D--	-H----LK--	-	-K-----
ANT70-NA.2	-------M-AAR--TQNNQ--------ALNI-	-K--AM.K--	-	MR--S-G
BCF06	--R----I--R.--T-NT--------TS-N--	-M-K-RGKI-	R	-AT--LR
HIV-1$_{MN}$	-AEEEVVIRSE.-FT-NA-T---H--ESVQIN-	T--NYNKRK	R	-H---GR

	—— V3		——		——>		
		25		37		CHARGE	
Consensus O	.WYSMG	L	E???NTK?SSR	?	AYC		?YNATDWEKALKQTAERYLE
ANT70.1	.----R	I	-R..TAEN---	A	---	(4)	E----N---I----------
ANT70.2	.----R	I	-R..TAEN---	A	---	(4)	E----N---I----------
ANT70-NA.1	.-----	-	-S..LPGN---	A	---	(3)	K-D-NE-V-I----------
BCF02	.-----	-	A-..GNGSE--	R	---	(2)	E--T-?-V-T----------
BCF07	.-----	I	-N..ENIPD--	K	---	(1)	---?-K-VE-----------
ANT70-NA.2	.R--VK	K	-R..TAGP..K	A	---	(7)	E----N---I----------
BCF06	.-V--A	A	KT.ESQNRE--	I	---	(7)	M--N-?-INT----------
HIV-1$_{MN}$	AF-TTK	N	I.....IGTI-	Q	-H-	(8)	NISRAK-NDT-R-IVSKLK-

Consensus O	LV.N?T?N?VT......M?FN?SS?GGDAEVTH?H
ANT70.1	--.-N-.GR-D......-T-SN--.---L----L-
ANT70.2	--.-N-.GR-D......-T-SN--.---L----L-
ANT70-NA.1	--.-N-.GS-N......-T--H--.---I-I--L-
BCF02	--.-N-.GNMS......-I--S-T.---L--DQL-
BCF07	--.-N-.GSIN......-T-GN-T.---P----L
ANT70-NA.2	--.-N-.DEAK......-T-AP--.---L--S-L-
BCF06	--.-K-.GNLSKTGNFSII--H-IS---V-ASSL-
HIV-1$_{MN}$	QFK-K-...........IV--Q--.---P-IVMHS

[a]Dashes (–) indicate concurrence with the Los Alamos HIV-1 type O consensus sequence. Dots (.) are introduced for obtaining the best alignment. Question marks (?) are introduced in the consensus O sequence to indicate variable positions. Positions 11, 25, and 37 of the V3 loop are boxed. Net positive charge of the V3 loop is indicated between parentheses ( ).

polymerase chain reaction (PCR), using a nested configuration. The reverse transcription primer and 3′ outer primer was 3′ELO-Not (5′ GCGCGGCCGCCCATATATCTTTCATATCTCCCCCT 3′; location 1407 to 1431) and the 5′ outer primer was 5′ELO-Not (5′ GCGCGGCCGCATTCCCATACACTATTGTGCTCCA 3′; location 625 to 648). For the nested PCR reaction the primers Sp6-5′CAM (5′ GATTTAGGTGACACTATAGTTACTTGTACACATGGCAT 3′; location 719 to 740) and T7-3′CAM (5′ TAATACGACTCACTATAGGGCAATAAAAGAATTCTCCATGACAG 3′; location 1126 to 1148) were used. The nucleotide location of the primer sequences are according to the HIV-1_{ANT70} isolate.[22] The nested PCR products were sequenced with Sp6 and T7 dye-labeled primers and analyzed on an automatic sequencer. Nucleotide sequences were aligned manually with the consensus sequences representing different HIV-1 subtypes as presented in the Los Alamos HIV Database.[22] The derived amino acid sequences of the seven isolates were compared with the HIV-1 group O consensus sequence and the alignment is presented in Table 1. Phylogenetic analysis was performed using the neighbor-joining method of the MEGA program[25] with a distance matrix input file generated by Kimura's two-parameter estimation.[26] The resulting phylogenetic tree is presented in Figure 1. The HIV-1 *env* tree consists of two major branches representing group M viruses and group O viruses and shows that all seven isolates included in this study belong to the group O branch. This analysis is supported by a bootstrap value of 100%. In addition, the analysis shows that the ANT70 and ANT70-NA isolates cluster together while the remaining isolates included in this study form discernable phylogenetic clusters.

To determine whether an association exists between replication and syncytium formation in MT-2 cells and the presence of positively charged amino acids in the V3 loop, the phenotype of the seven primary group O isolates was determined. Two million donor PBMCs and 1×10^6 MT-2 cells were infected with 1 ml of cell-free supernatant from each of the primary isolates. One day after infection the cells were washed

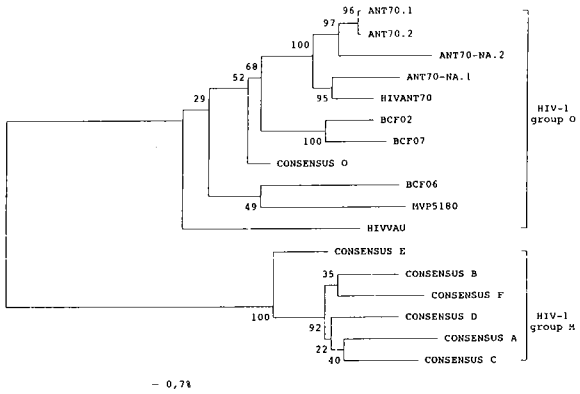

FIG. 1. Results of the phylogenetic analysis of a 339-nucleotide segment of the *env* region including the V3 loop (by the neighbor-joining method part of the MEGA program) for the seven patient isolates, the corresponding region of the O group isolates HIV-1_{ANT70}, HIV-$1_{MVP5180}$, and HIV-1_{VAU}, and the consensus sequences of the subtypes of the M group. The bootstrap values obtained for the cluster are given at the root of the cluster.

three times with phosphate-buffered saline (PBS) to remove residual virus inoculum and resuspended in culture media. The cultures were scored by p24 enzyme-linked immunosorbent (ELISA; Abbott, Abbott Park, IL) for virus replication and microscopically for syncytium formation. Viruses from each culture were harvested by centrifugation, filtered, and stored at −70°C. In addition, virus aliquots were stored in guanidinium isothiocyanate for subsequent RNA isolation. The p24 values and syncytium scores from the PBMC and MT-2 cultures after cell-free infection with the seven primary isolates are shown in Table 2. All primary isolates showed rapid p24 production in PBMCs, indicating that the inocula contained high doses of infectious virus. Syncytia were observed only in the ANT70-NA.2 and BCF06 PBMC cultures. These viruses also readily replicated and produced syncytia in the MT-2 cells. The remaining

TABLE 2. PHENOTYPING OF THE SEVEN HIV-1 GROUP O ISOLATES

	Virus production after infection (p24 in pg/ml)							*Phenotype*[a]			
	Day 5		*Day 13*					*PBMC*		*MT-2*	
Isolate	*PBMC*	*MT-2*	*PBMC*	*MT-2*	*Day 19 (MT-2)*	*Day 28 (MT-2)*	*Day 33 (MT-2)*	*p24*	*SI*	*p24*	*SI*
ANT70.1	260	0	3099[b]	0	0	0	0	+	−	−	−
ANT70.2	254	0	3397[b]	0	0	0	0	+	−	−	−
ANT70-NA.1	415	0	2216[b]	0	0	0	0	+	−	−	−
BCF02	>1000	0	3061[b]	0	59	730	460	+	−	+	−
BCF07	>1000	0	1595[b]	511	>1000[b]			+	−	+	−
ANT70-NA.2	>1000[b,c]	993[b,c]						+	+	+	+
BCF06	>1000[b,c]	732[b,c]						+	+	+	+

[a]Replication and syncytium formation in PBMCs and MT-2 cells are indicated by a plus symbol (+) in the p24 and SI columns, respectively.
[b]End of culture.
[c]Syncytium formation.

isolates did not induce syncytia in MT-2 cells even following prolonged cultivation.

Interestingly, two isolates (BCF02 and BCF07) replicated in MT-2 cells as indicated by p24 production, but did not induce syncytia. Viruses capable of replicating in MT-2 cells without the formation of syncytia is a rare but not unique observation[27] and suggests that MT-2 tropism and syncytium formation are independently determined properties of HIV-1 viruses.

On the basis of the characteristics observed in PBMC and MT-2 cultures, the seven HIV-1 group O isolates were divided into three categories: category 1, the NSI/non-MT-2 tropic isolates including ANT70.1, ANT70.2, and ANT70-NA.1; category 2, the NSI/MT-2 tropic isolates BCF02 and BCF07; and category 3, the SI/MT-2 tropic isolates ANT70-NA.2 and BCF06. To determine whether or not an association exists between the phenotype and sequence of HIV-1 group O isolates, a part of the envelope including the V3 loop of viruses obtained from PBMC cultures of the NSI/non-MT-2 tropic isolates and from MT-2 cultures of the NSI/MT-2 tropic and SI/MT-2 tropic isolates was sequenced. V3 loop sequences obtained from the phenotyped isolates were identical to the V3 loop sequences of the respective original isolates as shown in Table 1. Sequence alignment, in which HIV-1_{MN} is included to represent syncytium-inducing subtype B viruses, showed that the SI/MT-2 tropic HIV-1 group O isolates BCF06 and ANT70-NA.2 contain a positively charged amino acid residue at positions 11 and 25, respectively. The V3 loops of the SI/MT-2 tropic viruses otherwise had a high net positive charge (+7). This is in agreement with results obtained with viruses belonging to HIV-1 subtypes A, B, D, E, and F.[13,15–17] The two NSI/MT-2 tropic isolates BCF02 and BCF07 showed a positively charged amino acid at position 37 and both had a lower net positive charge than the NSI/non-MT-2 tropic isolates. Although the presence of a positively charged amino acid at position 37 (as mentioned in Table 1) is reported to be associated with T cell line tropism,[28] not all HIV-1 subtype E and F viruses that contain the positively charged amino acid lysine at this position are MT-2 tropic.[15,17] In addition, V3 loop sequences of proviral DNA obtained from PBMCs infected with four additional NSI/MT-2 tropic HIV-1 group O isolates (BCF01, BCF03, BCF08, and BCF11) had a low net positive charge and did not have a positively charged amino acid at position 37.[24] Thus, the relevance of a positively charged amino acid at position 37 and the role of the V3 loop in MT-2 tropism of HIV-1 group O viruses is questionable. Although it took 13 and 19 days to select the NSI/MT-2 tropic viruses from the primary BCF07 and BCF02 isolates, respectively, no sequence changes in the V3 loop were observed.

While the net positive charge of the V3 loop and, in particular, the presence of positively charged amino acids at positions 11 and 25 seem to be important for syncytium induction of group O isolates as well as most other HIV-1 subtypes characterized to date, the role of the V3 loop with respect to MT-2 tropism of HIV-1 group O viruses remains to be established. Despite highly divergent V3 loop sequences, the concordance between the association of V3 loop sequences and the syncytium-inducing phenotype observed among HIV-1 group M and group O isolates is indicative of the universal importance of the V3 loop to *env* properties.

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