Antibodies to V3 loop peptides derived from chimpanzee lentiviruses and the divergent HIV-1_{ANT-70} isolate in human sera from different geographic regions

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Objective: To study the spread of antibodies to V3 loop peptides of two chimpanzee lentiviruses and the divergent HIV-1_{ANT-70} isolate (group O) in human sera from different geographic regions, and to compare this with reactions to peptides from known North American (subtype B) and Zaïrean (subtype D) strains.

Methods: A total 2495 HIV-1-antibody-positive sera from nine countries were tested by enzyme-linked immunosorbent assay for antibodies to the V3 loop of 10 HIV/SIV isolates (including MN, SF2, HXB2, RF, MAL, ELI, Z6 and ANT-70 for HIV-1, and cpz-gab and cpz-ant for SIV).

Results: In each country, the highest prevalences were observed against the MN peptide (58.8–91.7%). Seroreactivity to other peptides from subtype B were generally lower. Prevalences of antibodies to V3 peptides derived from Zaïrean strains belonging to subtype D were generally lower than to subtype B. Relative high prevalences of sera reactive with the $SIV_{cpz-gab}$ V3 peptide were observed. The lowest rates were seen in Brazil (4.2%) and Belgium (25.7%). Among the African countries, the prevalence rates varied between 30.1 and 67.6%. Prevalence to the V3 loop derived from the $SIV_{cpz-ant}$ strains was much lower. Prevalence of sera reactive to the ANT-70 V3 loop peptide was very low, and the highest rates were observed in Cameroon (10.2%), Niger (6%) and Gabon (4.6%). Only the sera reactive to the ANT-70 V3 loop peptide from Cameroon and Gabon were confirmed on a specific HIV_{ANT-70} Western blot (i.e., presence of antibodies to the envelope protein gp120).

Conclusions: The extent to which different V3 peptide reactivity patterns reflect the circulation of different HIV-1 strains in a particular population is not yet clear. However, V3 peptide serology using the very specific V3 peptide of the HIV_{ANT-70} is a good indicator of the very aberrant group O in a particular population.

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Introduction

One of the problems in the development of an effective vaccine for AIDS is the genetic diversity of HIV-1. Numerous variant strains have been obtained from different geographic regions. At present, the HIV-1 virus group consist of five to eight genetic subtypes, depending on the coding sequence used [1–3]. Although this sequence heterogeneity is distributed throughout the whole genome, most is located in the *env* gene [4]. Most of the sequence variability found in gp120 is clustered in five hypervariable domains (V1–V5) [5–7]. The third variable region (V3) is a major target of vaccine research, because it contains an important neutralization site, and has been shown to bind and elicit isolate-specific neutralizing antibodies [8–11]. This segment of the envelope

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contains also B- and T-cell epitopes and plays a role in diverse functions such as infectivity, viral fusion and syncytium formation [12,13].

To develop a vaccine that is widely applicable, one must consider HIV-1 *env* gene variation. The principal approach to monitor diversity has been through genetic sequences, however, sequencing is expensive and laborious and therefore its use is limited. An alternative approach is serotyping which can be used to screen large population groups. Previous studies [14–16] on binding of serum from HIV-1-infected individuals to peptides, representing the highly antigenic HIV-1 neutralization epitope of the V3 loop of known sequences from diverse isolates, have shown this method to be relevant in studying antigenic and genetic diversity of HIV-1 [14–16]. This technique has also been used to provide epidemiologic information about the dynamics of the spread of some virus types in certain geographic areas [17].

We used this serologic approach mainly to study the spread of the two chimpanzee lentiviruses and one of the most aberrant HIV-1 strains (HIV-1_{ANT-70}; group O) in human sera from different geographic regions [18–22]. We compared the antibody reactions to these three divergent V3 peptides with reactions to peptides from known North American and Zaïrean strains.

Materials and methods

Patients and sera

A total 2495 sera positive for HIV-1 antibodies were collected and stored at 20°C. The sera had been screened for HIV antibodies at the time of collection with a commercial enzyme-linked immunosorbent assay (ELISA) kit, and positive samples were confirmed with a commercial Western blot (WB) or a recombinant protein/synthetic peptide line immunoassay (INNOLIA HIV-1+2, Innogenetics, Ghent, Belgium). Serum samples were collected from symptomatic and asymptomatic individuals in nine different countries: Brazil (n=163, 1989–1991), Belgium (n=379, 1989–1992), Côte d'Ivoire (n=380, 1990–1992), Niger (n=99, 1990–1991), Cameroon (n=185, 1991–1992), Gabon (n=188, 1989–1992), Zaïre (n=418, 1990–1991), Kenya (n=109, 1991–1992) and Rwanda (n=574, 1988–1990).

Peptides

The synthetic peptides were synthesized by Neosystems, Strasbourgh, France. The amino-acid sequences of the peptides are shown in Table 1.

Peptide assay

Antibodies to the V3 loop peptides were tested by ELISA. Stock solutions (1 mg/ml) of each peptide were reconstituted in 0.05 mol/l carbonate buffer (pH 9.6) and stored at 20°C. One hundred nanograms of peptides in 200 µl carbonate buffer were coated overnight at room temperature into each well of microtitre plates (NUNC, immunoplate, Copenhagen, Denmark). The plates were washed twice with wash solution [phosphate buffer solution (PBS), 0.01% merthiolate, 0.05% Tween 20] before use. Non-specific binding sites were blocked by incubation with 200 µl PBS containing 0.05% Tween 20 and 2% bovine serum albumin (BSA) at 37°C for 2 h. Serum dilutions (1/200) were made in blocking buffer and 200 µl was incubated with the immobilized peptides for 1 h at 37°C. After washing, antihuman immunoglobulin G peroxidase conjugate in blocking buffer was added and incubated for 1 h at 37°C. After washing, bound antibodies were visualized by adding substrate [8.7 mg tetramethylbenzidine/ml dimethylsulphoxide (DMSO) diluted 1/100 in 0.1 mol/l citrate phosphate buffer, pH 4.3 and 0.006% H_2O_2]. The reaction was stopped by adding 50 µl sulphuric acid and the optical densities (OD) were read at 450 nm. Ten negative sera from the corresponding country were used to establish the cut-off value of each assay, which was the mean of the OD of the negative sera plus three SD. Cutoff values calculated in this manner ranged from 0.15 to 0.5. For uniformity and to eliminate non-specific reactivity the highest cut-off value (0.5) was used for all the assays [15,22].

Western blot (WB)

WB were prepared from the HIV-1_{ANT-70} and the $SIV_{cpz-gab}$ viruses as described previously [21].

Table 1. Amino-acid sequences of V3 peptides.

Peptides	Subtype	Amino-acid sequences
HIV-1		
MN	B	C N K R K R I H I G P G R A F Y T T K N
SF2	B	. . T . . S . Y . . . . H . . G R
HXB2	B	. . T . . Q R Q R . . . . . . V . I G K
RF	B	. . T . . S . T L . R . . V I . A . G Q
MAL	D	. . T . R G . . F . . . Q . L . . . G I
ELI	D	. . T . Q . T P . . L . Q S L . . . R S R S
Z6	D	. . T . Q S T P . . L . Q . L . . . R G T K
ANT-70	O	. D I Q E M R . . . M A N Y S M G I G G T A G N S
SIV_{cpz}		
gab	O	. . T . G E V Q . . . . M T . . N I E . V V G D
ant	O	. R T C R N L N . . . . M T . . N V Q I A T G D

Results

Prevalences of antibodies to the different peptides

The prevalences of antibody reaction to different V3 peptides in the different countries are summarized in Table 2. In each country the highest antibody reaction was observed against the HIV-1_{MN} peptide (subtype B), although prevalences were different and ranged from 91.7 to 58.8%, with the lowest prevalence in Brazil. The second most recognised peptide was SF2, also belonging to subtype B. Antibody binding to the two other peptides from the same B subtype (HXB2 and RF) were low overall in all countries.

Prevalence rates of antibodies to the peptides derived from Zaïrean strains belonging to subtype D were generally lower. Antibodies to this subtype were almost absent in sera from Brazil, and the OD were low (OD < 1.5 or OD/cut-off ratio < 3).

Relative high prevalences were seen for the V3 loop peptides derived from the chimpanzee lentiviruses, especially for the peptide from the $SIV_{cpz-gab}$ strain, isolated from a chimpanzee in Gabon. The lowest rates were seen in Brazil (4.2%) and Belgium (25.7%). Among the African countries, the highest prevalences were observed in West Africa and the lowest in the East African country of Kenya (Table 2). Prevalences to the V3 loop derived from the $SIV_{cpz-ant}$ strain were much lower. Most of the sera (91%) reacting with the $SIV_{cpz-ant}$ peptide reacted simultaneously with the $SIV_{cpz-gab}$ peptide; the remainder (9%) had only weak antibody reactions to the $SIV_{cpz-ant}$ peptide.

Prevalences of sera reactive to the V3 loop peptide derived from the aberrant strain $HIV1_{ANT-70}$, from Cameroon, were very low. The highest rates were observed in Cameroon (10.2%), Niger (6%) and Gabon (4.6%). Reactions with high OD (>2.5) were only seen in Cameroon and Gabon, two neighbouring countries, where reactions were very specific to this peptide.

Simultaneous antibody response to various peptides and to different subtypes

As can be seen from the data on the prevalences to each individual peptide (Table 2), the majority of the sera reacted with more than one peptide. The amount of sera reacting simultaneously with different peptides varies for each country (data not shown).

Among the 2495 sera tested, 2439 were analysed further for the possible combinations of antibody patterns to the three major subtypes (subtypes B and D, and SIV_{cpz}). Sera reactive with one or more V3 peptides of HIV-1 isolates from subtype B were observed most frequently (31.8%).

Overall, 1007 sera (41.3%) had antibodies to the peptides from subtype D, among which 22 reacted only with these peptides. All others also showed a reaction to one or more peptides from subtype B.

Of all the sera tested, 905 (37.1%) out of 2439 reacted with at least one of the two SIV_{cpz} peptides. Among these, 319 (35.2%) reacted simultaneously with subtype B peptides, only 23 (2.5%) with subtype D and 524 (57.9%) with both. Thirty-nine sera reacted only with SIV_{cpz} peptides.

Antibody reaction to HIV-1_{ANT-70} peptide in combination with other genetic subtypes

On the 2495 sera tested, 81 (3.2%) had antibodies to the HIV-1_{ANT70} peptide. We subdivided these sera into three groups according to OD and examined the number of simultaneous reactions with other peptides. The majority of the sera (61 out of 81) reacting with ANT-70 had low antibody reactions (OD < 1.5); only six (9.8%) of these reacted with the HIV-1_{ANT-70} peptide only or in combination with one subtype only, versus five (55.5%) out of nine, and nine (81.8%) out of 11 in the groups with intermediate (1.5 < OD < 2.5) and high (OD > 2.5) antibody response, respectively. A high OD with the HIV-1_{ANT-70} peptide indicates a more specific reaction.

Table 2. Percentages of sera reacting with the different V3 peptides in different geographic locations.

Peptide	Brazil (n = 163)	Belgium (n = 379)	Côte d'Ivoire (n = 380)	Niger (n = 99)	Cameroon (n = 185)	Gabon (n = 188)	Zaïre (n = 418)	Kenya (n = 109)	Rwanda (n = 574)	Total (n = 2495)
HIV-1										
MN	58.8	88.8	91.7	89.8	85.9	73.3	87.7	73.3	83.9	84.0
SF2	34.8	78.2	75.1	84.7	70.1	48.7	68.7	53.2	57.4	65.0
HXB2	5.5	10.4	11.0	15.1	5.4	12.6	16.0	2.7	18.1	11.4
RF	10.9	30.6	5.7	22.1	16.6	7.9	15.6	4.5	8.3	13.3
MAL	0.6	17.2	14.9	39.3	15.1	22.7	39.7	22.0	22.4	23.6
ELI	3.7	5.2	17.4	35.3	10.7	12.7	17.7	6.4	8.3	12.1
Z6	2.4	25.6	40.1	48.3	39.4	27.0	40.5	38.5	31.5	32.3
ANT-70	0.0	0.8	1.2	6.0	10.2	4.6	2.5	0.0	5.0	3.2
SIV_{cpz}										
ant	0.0	2.2	5.4	6.0	2.6	4.8	6.6	2.8	6.4	4.7
gab	4.2	25.7	51.3	67.6	41.5	41.3	38.1	33.1	43.0	38.6

Relation between antibody response to HIV-1_{ANT-70} and $SIV_{cpz-gab}$ peptides and gp120 antibodies on WB

Thirty-six sera with antibodies to the HIV-1_{ANT-70} peptide and eight sera with antibodies to the $SIV_{cpz-gab}$ peptide only were tested on in-house WB strips from the corresponding viruses. The sera were subdivided in different categories according to their OD. All eight sera with OD > 2.5 had antibodies to the whole gp120 of the HIV-1_{ANT-70} virus on WB, versus three out of six sera with OD between 1.5 and 2.49, and three out of 16 sera with OD between 0.5 and 1.49. These three sera had antibodies to HIV-1_{ANT-70} peptide only. None of the six sera without antibodies to the HIV-1_{ANT-70} peptide reacted with the gp120 (Table 3).

One of the eight sera with antibodies to the $SIV_{cpz-gab}$ peptide only and with OD > 2.5 showed a weak antibody reaction to the gp120 protein of the corresponding virus on WB.

Table 3. Number of sera with antibodies to gp120 outer membrane protein of HIV-1_{ANT-70} according to optical densities (OD) of reactions to the ANT-70 V3 peptide in enzyme-linked immunosorbent assay (ELISA) in the different countries.

OD to ANT-70 V3 peptides in ELISA	No. tested	No. positive with ANT-70 gp120 in Western blot
OD ≥ 2.5		
Cameroon	7	7
Gabon	1	1
Total	8	8
1.5 ≤ OD < 2.5		
Cameroon	2	1
Gabon	2	2
Rwanda	1	0
Zaïre	1	0
Total	6	3
0.5 ≤ OD < 1.5		
Cameroon	6	0
Gabon	3	0
Rwanda	1	0
Niger	3	0
Zaïre	1	0
Belgium	2	0
Total	16	3
OD < 0.5	6	0

Discussion

In each country, the highest prevalences were observed against the HIV-1_{MN} peptide, belonging to subtype B. The prevalence of 88.8% in Belgian sera is comparable with the 79–100% observed in the United States and Europe [14,23–25].

As described previously for East African countries [14,25], our study showed that in West and Central African countries the prevalences to the HIV-1_{MN} peptide were high. Seroreactivity to other peptides from subtype B were generally lower as observed in previous studies [14,23].

Prevalences of antibodies to V3 peptides derived from Zaïrean strains belonging to subtype D were generally lower than to subtype B. The highest reactivities were observed in sera from Africa, although no significant differences were observed between East, Central and West African countries. Interestingly, antibodies to these peptides are almost always associated with antibodies to subtype B.

Surprisingly, high amounts of sera had antibodies to the peptides derived from the chimpanzee lentiviruses, especially to the $SIV_{cpz-gab}$ V3 peptide. In analogy to the subtype D peptides very low reactions were seen in Brazil. For the Belgian sera a link with Africa or Africans was documented (data not shown), also indicating that these are peptides with an African origin. This was also the case for reaction with subtype D peptides in Belgian sera.

Only 3% of sera reacted with the HIV-1_{ANT-70} peptide, among which only a small percentage reacted to the HIV-1_{ANT-70} peptide only. This peptide seemed to be more specific than the others tested, especially when high OD were observed. From our results, this virus seemed to be located in two neighbouring Central African countries, Gabon and Cameroon, and confirms data of a previous preliminary study [26]. In support of our findings, a new HIV-1_{ANT-70}-like virus (MVP5180) has been recently isolated from a Cameroonian HIV-positive patient [27]. For two sera reactive with the HIV-1_{ANT-70} peptide and gp120 on HIV-1_{ANT-70} WB, we sequenced a 280 base-pair fragment in the *pol* region of the corresponding virus. Phylogenetic analysis showed that these viruses cluster with HIV-1_{ANT-70} and HIV-$1_{MVP5180}$ [28].

Overall, about 10% of the HIV-antibody-positive sera tested had no antibodies to any of the 10 V3 peptides, giving an indication that these individuals were probably infected with different HIV-1 strains. This is suggested by different studies from Brazil where V3 sequences from Brazilian strains are often different from those observed in the US [29–31]. High prevalences of sera non-reactive with any of the V3 peptides studied are not sufficient alone to indicate presence of aberrant or divergent HIV-1 strains in a certain region. In Cameroon for example, where only 5.4% of the tested sera were negative (data not shown), we observed the highest prevalences of reactivities with the very aberrant HIV-1_{ANT-70} peptide.

In conclusion, peptide serology using V3 peptides of HIV-1 isolates belonging to different subtypes helps to rapidly document the existence of different V3 peptide reactivity patterns in different geographical localities. The extent to which these patterns reflect the circulation of different HIV-1 strains in a particular population is not yet clear due to the simultaneous reactions with peptides from different strains and subtypes. The relevance of human sera containing antibodies reactive to the SIV_{cpz} peptides in ELISA needs to be further

clarified by sequence data. The large number of sera that reacted simultaneously with other peptides indicate that these are more likely to be crossreactions than specific antibody responses. On the other hand, reactions with the HIV-1_{ANT-70} peptide are more specific and serology using the V3 peptide of HIV-1_{ANT-70} can increase the chance of finding viruses belonging to subtype O in a particular population.

Studying the spread of HIV-1 viruses from group O is important since some of their sera react negatively in some HIV-screening assays and produce indeterminate WB patterns [32]. Studying the spread of these viruses will give an indication as to whether the present guidelines and strategies for blood screening and serodiagnosis need modification.

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