

SPECIAL REPORT

Determination of HIV-1 Subtypes in Hungary by Synthetic Peptides Representing the V3 Loop of *env*

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For the determination of HIV-1 diversity and serotyping of HIV-1 subtypes, an enzyme immunoassay was developed based on synthetic peptides representing immunodominant epitopes of the V3 loop of HIV-1 subtypes A, B, C and E, respectively. Sera from 53 asymptomatic HIV-1 infected individuals were tested for their pattern of binding reactivity to the synthetic peptides. 45/52 (85%) of the sera reacted exclusively to V3 peptide representing HIV-1 B subtypes, 4/52 (7.6%) of the sera showed

cross reactivity to A/B peptides and 1/52 (1.9%) of the sera reacted with both A and C peptides. No single reactivity with subtype A or E peptides have been observed. Results together with nucleotide sequence analysis of the V3 region of clinical isolates suggest that HIV-1 infection in Hungary has been induced predominantly by strains belonging to HIV-1 subtype B. (Pathology Oncology Research Vol 2, No 4, 268–271, 1996)

Key words: HIV-1, subtypes, Hungary

Introduction

Human immunodeficiency virus type 1 (HIV-1) classification is based on the variation in the nucleic acid and amino acid sequences of the virus isolates from various geographical locations. HIV-1 M group strains have so far been classified into nine genetic subtypes or clades, designated A through I, based on phylogenetic analyses of *env* or *gag* nucleotide sequences.^{9,10} The major neutralization domain of HIV-1 is a loop (V3), situated in the third variable domain of the external envelope glycoprotein (*env* gp 120, or SU).⁸ V3 consensus sequences from each subtype have been described.¹⁰ Peptides synthesized to represent V3 loop consensus sequences of HIV-1 subtypes are all antigenic for HIV-1 seropositive human sera.

Hungary is a landlocked country in Central Europe. The AIDS epidemic has started years later than in Western Europe. The first serologically confirmed cases were reported in 1985 (Nagy K. personal communication), and cases meeting the CDC definition of AIDS were diagnosed in 1986. In

1996, AIDS morbidity is still 2/100,000 inhabitants.¹⁹ Importantly, a comprehensive testing program, through which more than 1.8 million samples have been analyzed to date, provides assurance that the prevalence of infection is not underestimated due to underreporting. The delayed occurrence and still low rate of the AIDS epidemic raises the possibility of the presence of HIV subtypes with altered replication capacity and pathogenicity compared to those strains of HIV detected in Western Europe and the USA.

To determine HIV-1 diversity and serotyping HIV-1 strains, we developed a V3 peptide-based enzyme immunoassay (EIA). We report here the identification of the dominant HIV-1 subtypes serologically using a panel of V3 peptides (A, B, C, and E). Serum samples from studied subjects were tested for their binding reactivities to these peptides. The serological data have been compared with genotypic and sequencing data. This technique offers a simple and inexpensive means to elucidate the epidemiology of the spread of HIV-1 in Hungary.

Materials and Methods

Synthetic V3 peptides

A panel of four 10-mer synthetic V3 peptides was generated representing the main immunodominant regions of *env* gp120 of HIV-1 subtypes A, B, C, and E. Sequences

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were derived from the consensus of the V3 sequences of the HIV-1 subtypes, respectively.¹⁰

Peptides were synthesized by a solid-phase method using Fmoc chemistry on an Applied Biosystem synthesizer, and purified by high performance liquid chromatography. Details of the amino acid sequences of the V3 peptides, containing characteristic epitope motifs (GPGR/GPGQ) are shown in *Table 1*.

Table 1. Amino acid sequences of the V3 peptides representing HIV-1 subtypes

HIV-1 subtype	Synthetic decapeptides
A	SVHIGPGQAF Ser-Val-His-Ile-Gly-Pro-Gln-Ala-Phe
B	SIHIGPGRAF Ser-Ile-Ile-Ile-Gly-Pro-Gly-Arg-Ala-Phe
C	RIRVGPGQTV Arg-Ile-Arg-Val-Gly-Pro-Gly-Gln-Thr-Val
E	SITIGPGQVF Ser-Ile-Thr-Ile-Gly-Pro-Gly-Gln-Val-Phe

Patients sera

Serum specimens from 52 asymptomatic HIV-1 infected individuals belonging to the homosexual and/or STD risk groups of AIDS were analyzed. Serum samples were collected in 1995 by random sampling. All sera tested positive for antibodies to HIV-1/2 using commercial ELISA (Organon Teknika HIV-1/2 Uniform II).

Virus isolation and determination of HIV-1 provirus

Infectious HIVs were isolated from peripheral blood mononuclear cells (PBMC) directly, as well as by cocultivation.¹¹ Replication capacity and syncytium induction of the isolates were determined in PBMCs; in cell lines of lymphoid origins such as Jurkat tat-3, CEM and MT-2; and of macrophage origin, such as U937.^{12,13} In testing for the presence of HIV-1 provirus, PBMCs of HIV infected individuals, as well as cells infected by the isolates, were analysed by PCR using *gag* specific primers followed by DNA hybridisation. DNA sequencing of *env* V3 loop encoding regions from certain Hungarian clinical isolates was determined with PCR amplification of a 211 nucleotide fragment of HIV-1 *env*. Nucleotide alignment comparison was made with HXB-2, HIV-1 BRU(LAI) and HIV-1 NL43(NY5). Details of PCR and Southern blot DNA hybridisation, as well as sequence alignments, have been described elsewhere.^{12,13,14}

HIV-1 V3 peptide serology

HIV-1 antibody binding to the V3 loop peptides was measured by an indirect ELISA using synthetic decapeptides representing immunodominant epitopes of HIV-1

subtypes A, B, C and E, respectively. Synthetic peptides were solubilized in 0.1 M carbonate-bicarbonate buffer (pH 9.6) to a final concentration of 20 µg/ml. Microtiter plate (Greiner) wells were coated directly for 48 hours at room temperature with 100 µl/well of peptide solution. After washing, plates were blocked with blocking buffer (PBS pH 7.4 containing 5% BSA) and kept at +4 °C in PBS until use. Test sera were diluted in serum diluent (blocking buffer containing 0.01% Tween 20) at 1:100 dilution, and incubated with the immobilized peptides at 37°C for 60 min. The plates were washed with washing buffer (PBS with 0.05% Tween 20) and antibody bound to the peptides was detected by anti-human IgG peroxidase conjugate (Sigma) at a dilution of 1:2000 after an incubation for 1 hr at 37°C. Antigen-antibody complexes were detected by adding tetramethylbenzidine (TMB)/H₂O₂ substrate (Organon Teknika) and the reaction was stopped by adding 1M H₂SO₄. Optical density (OD) was measured spectrophotometrically at 450 nm. The cut-off value (CO) of the assay was calculated (means of HIV-1 negative samples + 3SD/x2). The highest ratio (OD/CO) of antibody binding to a V3 peptides was determined and serotype/subtype was identified accordingly.

Results

Sera from asymptomatic HIV-1 infected individuals were tested for their pattern of binding reactivity to the synthetic peptides representing HIV-1 subtypes A, B, C and E, respectively. Results are expressed as the ratio of antibody binding - measured in optical density (OD) units - to the individual peptide, to the cut-off value (CO) of the HIV-negative control sera, (OD/CO).

Altogether sera of 52 HIV-infected individuals were tested. This represents more than ten per cent of all identified cases of HIV infection in Hungary. 45/52 (85%) of the sera tested showed antibody binding exclusively to V3 peptides representing HIV-1 B subtype (*Table 2*). 4/52 (7.6%) of the sera showed cross reactivity to A/B peptides, and 1/52 (1.9%) of the sera reacted both A and C peptides.

Table 2. Binding of sera of HIV-1 infected persons to V3 peptides representing env V3 region of HIV-1 subtypes A,B,C and E_a

	V3 peptides ^b						
	A	A/B	A/C	B	C	E	NR
serum samples (52)	0	4	1	45	0	0	2
(%)	0	7.6	1.9	85	0	0	3.8

a. Sera from HIV-1 infected persons were tested in ELISA, where synthetic peptides representing V3 region of HIV-1 subtype A, B, C and E were used as antigen

b. All sera were tested for binding to V3 peptides A,B,C and E. Some sera cross-reacted with more than one peptides.

NR - non reactive

No reactivity with subtype A or subtype E peptides have been observed. 2/52 (3.8%) of the sera reacted with neither of the V3 peptides used, and so could not be serotyped because of a lack of peptide binding (Table 2.).

Based on the nucleotide sequencing of HIV-1 *env* V3 loop encoding regions of virus isolates from some of the infected people, whose sera were also tested in V3 peptide ELISA, these results suggest that serological antigenicity is related to genotype. In a representative comparison, an amplified V3 region (1-134 bp) of a clinical isolate *HIV-1 Hun610* showed a high similarity (95 - 96.9%) to the V3 region of *i.*) HIV-1 BRU(LAV) (6692-6823), *ii.*) HIV-1 HXB-2 (7098) and *iii.*) HIV-1 NL43/NY5/ (7089-7220). All are cloned isolates representing HIV-1 subtype B.

Discussion

We developed a peptide EIA for serologic typing of HIV-1 infections in Hungary that is based on the V3 sequences of four major HIV-1 variants. It has been demonstrated that V3 peptide serology may be used for the prediction of HIV-1 genotypes, and this can be applied to the investigation of the distribution of HIV-1 diversity in the population.

HIV-1 subtypes could be analyzed by direct sequencing of PCR amplified, or cloned, V3 sequences.^{2,13,20} Another effective method is the heteroduplex mobility assay.⁵ Based on earlier reports using serological typing,^{4,15} we have also developed an assay to determine HIV-1 subtypes, based on the V3 epitope. V3 antigen serves as an important functional and biological domain including neutralization, cell tropism and syngeneity inducing capability.^{6,16,17} Moreover, the V3 loop is strongly antigenic, and antibodies to the V3 epitope have been associated with protective immunity and neutralizing activity.^{1,3,7,18}

The results based on V3 epitope serotyping suggest that HIV-1 infection in Hungary has been induced predominantly (>85%) by strains classified as HIV-1 subtype B. This has been supported by sequencing data showing a high similarity of V3 sequences in clinical isolates to that of cloned prototypes of HIV-1 subtype B strains, i.e. HIV-1 BRU(LAV). Although most sera could be typed by this peptide EIA, 3.8% failed to react with peptides representing subtypes A,B,C and E. This could be due to *i.*) some patients not producing antibodies to the V3 loop peptides, *ii.*) direct adsorption of synthetic peptides onto a solid phase distorting the binding site, or *iii.*) the amino acid sequence of the V3 peptides lacking universal recognition, as they are derived from consensus rather than real virus isolates. Peptides A, C and E all share a common GPGQ motif at the centre of the molecule, while peptide B has a GPGR motif. Since these four amino acids are required for antibody binding,¹⁵ antibodies to an epitope containing the GPGR might not recognize the GPGQ motif.

An EIA using synthetic peptides from the V3 region of gp120 can be a simple and inexpensive method for large scale serotyping of HIV-1 infections in geographic areas where HIV genetic variability is limited and well characterized. It can provide useful epidemiologic information about the dynamics of the spread of the virus. The V3 based classification and the biological characterization of HIV subtypes and knowledge of their global incidence and prevalence may be useful in designing appropriate HIV vaccines. This may lead to better understanding of the geographical distribution and functional significance of diversity in relation to the transmission and pathogenesis of HIV.

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