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V3 Peptide Enzyme Immunoassay for Serotyping HIV-1 Infected Pregnant Thais

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SUMMARY **Previous molecular epidemiological studies show that at least 2 subtypes of HIV-1 circulate in Thailand. HIV-1 subtype B or Thai genotype B was associated with an early epidemic and was prevalent in intravenous drug users. Meanwhile, HIV-1 subtype E or Thai genotype A was becoming widespread among heterosexuals. We studied the HIV subtypes of 161 HIV-1 seropositive pregnant women. Of these, 143 pregnant patients (88.8%) tested positive for subtype E alone and 8 women (5.0%) had evidence of infection with subtype B alone. There was serologic evidence of infection with a mixture of subtypes in 7 women while the infecting subtype could not be identified in the remaining 3 women. This result agrees with previous information that subtype E predominates in Thai heterosexuals.**

Understanding the epidemiology of the HIV pandemic has been facilitated by the development of the polymerase chain reaction (PCR).[1] PCR can amplify HIV nucleic acid from infected patients and permits the identification of the infecting viral strain.[2] Eight subtypes of HIV-1 have been identified; designated A through F, H and M.[3] Subtype B predominates in Europe, the Western hemisphere, Japan, Thailand and Australia.[4] Subtype C has been found mostly in Southern Africa, the Central African Republic and India. Subtype E was first identified in Thailand and recently found in the Central African Republic. Subtype F has been found in Romania[5] and is a rare variant in Brazil. Isolates from Gabon and the Russian Federation were designated subtype H. An "outlier" subtype O containing two human and two chimpanzee isolates has been identified in Cameroon and Gabon.[6]

McCutchan *et al.*[7] in 1992 reported two distinct HIV-1 variants found in Thailand. One variant (the Bangkok variant) resembled those prevalent in North America and Europe and another variant (The Northern Thailand variant) was unlike any subtype previously described. These data were based on env and gag nucleotide sequences and PCR fingerprinting. Ou *et al.*[8] in 1992 and 1993 found that two distinct genetic subtypes (presently named E and B, respectively) of HIV-1 were circulating in Thailand and apparently segregated by mode of transmission.[8] Subtype E predominated in people infected sexually while subtype B was found mainly in injecting drug users. Both subtypes B and E contain GPGQ tetrapeptide at the top of the V3 loop.

We studied 161 serum specimens collected from HIV-1 seropositive pregnant women. Rapid HIV-1 subtyping was performed using a V3 peptide enzyme immunoassay (EPA). This technique offers a simple and inexpensive means of elucidating the epidemiology of the rapid spread of HIV-1 in Thailand.[9]

SUBJECTS AND METHODS

Subjects

Serum specimens were obtained from HIV-1 seropositive pregnant patients who attended the antenatal

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care clinic at Siriraj Hospital from September 1992 to June 1994. All sera were positive for HIV-1 by Serodia-HIV (Fujirebio, Japan), Wellcozyme HIV-1+2 (Murex, England) and Western blot analysis (Bio-Rad, Hercules, California, USA). Sera were stored at -35°C until tested.

Table 1. HIV-1 subtypes in HIV-1 infected pregnants by peptide EIA.

Peptides	No. of reacted specimens (%)	Subtypes identified by peptide EIA
PND-A*	143 (88.82%)	E
PND-B/Q*	4 (2.48%)	B
PND-MN*	4 (2.48%)	B
PND-A and PND-B/Q**	5 (3.12%)	E and B
PND-A and PND-MN**	1 (0.62%)	E and B
PND-A, PND-B/Q and PND-MN**	1 (0.62%)	E and B
PND-B/R*	0 (0.00%)	-
Non-reactive***	3 (1.86%)	Untypable

*Specimens reactive with only one of the foreign peptides.
**Specimens cross-reactive with the foreign peptides.
***Specimens non-reactive with the foreign peptides.

Synthetic peptides

Four peptides were used from gp120, the principal neutralizing determinant of HIV-1. Each peptide was 14 amino acids long and they were designated PND-A, PND-B/Q, PND-B/R and PND-MN. Peptides PND-A (TSITIGPGQVFYRT), PND-B/Q (KSIHLGPGQAWYTT) and PND-B/R (KSIHLGPGRWYTT) were derived from the consensus sequences of Thai genotypes A and B, respectively, as reported previously.[8] Peptide PND-MN (KRIHI GPGRAFYTT) was derived from HIV-1 MN,[7] a typical North American-European variant. Peptides were synthesized by a solid-phase method using FMOC chemistry on an Applied Biosystems Model 431-A synthesizer (Foster City, California, USA) according to the manufacturer's protocol, and were partially purified by reverse-phase high performance liquid chromatography.[9]

Peptide EPA

Lyophilized peptides were solubilized in 0.1 M carbonate-bicarbonate buffer (pH 9.6) to a final concentration of 10 μg/ml for PND-B/Q, PND-B/R and PND-MN and 5 μg/ml for PND-A (because of its lower solubility). Immulon II microtiter plate wells (Dynatech Laboratories, Chantilly, Virginia, USA) were coated overnight at 4°C with 100 μl/well of peptide solution. The microtiter plate wells were washed once with 0.01 M phosphate-buffered saline containing 0.05% Tween 20 (PBS/Tween, pH 7.2) and then blocked with mild buffer (5% non-fat dry milk in PBS with 0.3% Tween 20) for 2 hours at 37°C. Coated plates were washed four times with PBS/Tween, dried, sealed, and stored desiccated at 4°C until use. Diluted test sera (100μl of 1:2,000 dilution in milk buffer) were added to the wells and incubated for 1 hour at 37°C. Unbound antibodies were removed by five washes with PBS/Tween. Goat anti-human immunoglobulin G (IgG) peroxidase labeled (Bio-Rad); 100μl/well, 1:2,000 dilution in milk buffer) was added and incubated for 1 hour at 37°C. After five washes with PBS/Tween, antibody complexes were detected by adding 100 μl/well of 3,3′, 5,5′, tetramethylbenzidine (TMB) H_2O_2 substrate (Bio-Rad). The reaction was stopped by adding 100 μl/well of 1 M H_2SO_4. Absorbance was read at 450 nm; and a cut-off value of 0.3 was chosen-approximately equal to the mean absorbance of the negative controls plus 7 SD.

RESULTS

The results of peptide serotyping are shown in Table 1. Subtype E was the most common subtype (88.8%) in these pregnant women; only 8 (4.9%) were infected with subtype B.

DISCUSSION

Available HIV-1 envelop sequences from major centers of the HIV pandemic have defined a minimum of eight distinct genetic subtypes:- A, B, C, D, E, F, G and H. Recently, HIV-1 subtype O was reported.[6] In this study, serotyping of HIV-1 positive pregnant women presenting to an antenatal clinic in Thailand found that 89% of HIV infections were by subtype E. This subtype has been shown previously to be the predominant type transmitted heterosexually in Thailand.[10] Most sera could be typed by the peptide EIA but 2% failed to react. This could be due to failure of some pregnant women to produce antibodies to V3 loop peptides, the presence of minor variants of tested subtypes, or to the existence of other minor viral subtypes not yet characterized. A higher proportion of subtype E sera cross-reacted with peptide B/Q than to peptide MN. Peptide B/Q shares a common GPGQ motif at the center of the molecule, while the MN strain of HIV-1 has a GPGR motif. These four amino acids are required for antibody binding. Antibodies to

an epitope containing the GPGQ motif might not recognize the GPGR motif of the MN strain. No sera reacted with peptide B/R, indicating that HIV-1 strains with a V3 loop containing the GPGR motif are not prevalent in Thai pregnant women. More information on genetic diversity and virus characterization is required to explain dual infections and non-reactive sera.

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DATE	:	8-11 December 1998
VENUE	:	Philippine International Convention Center Manila, Philippines
LANGUAGE	:	English
OFFICIAL HEADQUATER HOTEL	:	Westin Philippine Plaza Hotel
PRELIMINARY TOPICS	:	Respiratory allergies Atopic dermatitis, contact dermatitis, urticaria, skin allergies Occupational allergies Pediatric allergies Molecular biology, allergic inflammation Pharmacotherapy Autoimmune diseases, connective tissue disorders Immunotherapy Immunology of tropical disorders (malaria, dengue, TB, hepatitis, AIDS)
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