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APASARA MUDNGOEN: GENOTYPIC AND BIOTYPIC CHARACTERIZATION OF HIV-1 SUBTYPE E ISOLATED FROM HIV-1 INFECTED DISCORDANT AND CONCORDANT COUPLES. THESIS ADVISOR: RUENGPUNG SUTTENT, Ph.D., M.D., PRASERT AUEWARAKUL, M.D., Dr. Med., WANNEE KANTAKAMALAKUL, Ph.D. 177 p.
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HIV-1 subtype E was rapidly expanded thoughout the century, becoming the predominant subtype in Thailand. Several groups reported cases or series of persons either presumptively or definitely exposed to HIV-1 in whom subsequent evidence of sustained HIV-1 infection did not occur. The present study investigated viral factors and host factors that influenced lower transmission in Highly Exposed Persistently Seronegative (HEPS) groups. HEPS subjects consisted of 15 males (HH01, HH02, HH11, HH13, HH16, HH17, HH18, HH31, HH33, HH35, HH39, HH43, HH45, HH49, and HH55) and 2 females (HW48 and HW53). The HIV-1 infected partners of HEPS consisted of 15 female (PW11, PW35, PW45, PW49, PW55, PW01, PW02, PW13, PW16, PW17, PW18, PW31, PW33, PW39, and PW43) and 2 male (PH48 and PH53) and 5 HIV-1 infected concordant couples (CH06, CW06, CH07, CW07, CH34, CW34, CH57, CW57, CH59 and CW59). All subjects were asymptomatic at the time of study. EDTA blood was collected from both HIV-1 infected – and HEPS subjects. HEPS confirmed eligibility by HIV-1/2 ELISA and PBMC DNA PCR with *gag/pol* gene. None of them were infected. Mean viral load of HIV-1 infected partners of HEPS was log 4.20 ± 0.88 copies/ml and that of concordant couples was log 4.32 ± 0.45 copies/ml. No significant difference was found between these two groups.

To access any HIV-1 genetic abnormality presented in HIV-1 infected partners of HEPS, nucleotide sequencing of *nef* gene and LTR was performed on HIV-1 proviral DNA and genomic RNA of 17 HIV-1 infected partners of HEPS and 5 HIV-1 infected concordant couples. Four deletion patterns of *nef* gene were found in this experiment. Point mutations in *nef* gene and LTR region were found.

HIV-1 biological phenotypes were studied from 6 HIV-1 isolates from partners of HEPS and 7 isolates from concordant couples. Of 6 isolates from HIV-1 infected partners of HEPS, 3 isolates (PW33, PW49 and PH53) were M-tropic viruses, one (PW55) was T-tropic virus and 2 isolates (PW11 and PW45) retained both M-tropic and T-tropic (dual tropic) viruses. Of 7 isolates from concordant couples, 3 isolates (CW07, CH57 and CH59) were M-tropic viruses, two (CW33 and CW56) were T-tropic viruses and the last two isolates (CW34 and CH34) were dual tropic viruses.

The capability of viral infection was compared between HEPS PBMC and normal donor PBMC. Neither PBMC from HEPS nor those from normal donors were different in supportive infection of the viruses.

Neutralizing activity of NPO3 HIV-1 subtype E lab strain was relatively sensitive to neutralization by multiple plasma. All plasma from partners of HEPS except PH48 and PH53 contained neutralizing activities against lab strain subtype E (NP03) less than those of plasma from concordant couples and pool plasma. Viruses from partners of HEPS and discordant couples were not found any difference of the sensitivity to neutralization. However, PW55 isolates from partner of HEPS was resistance of neutralization by all of tested plasma. Plasma of these two concordant couples showed more broadly neutralization against primary isolates than those of partners of HEPS.

To determine nucleotide sequence of R5 gene, whose protein act as the secondary receptor of HIV-1 to enter the cell. No mutation sequence was detected in these HEPS R5 gene.

In conclusion, No significant difference of mean viral load was found between HIV-1 infected partners of HEPS and concordant couples. Viral factors found in partner of HEPS were *nef* gene deletion and point mutations in *nef* gene and LTR region. There was no difference in replication pattern of HIV-1 isolates in each kind of biotype between these two groups and NT activity was not different between these groups. No mutation sequence was detected in R5 gene of their HEPS.

These results suggest that the *nef* deletion and point mutations in *nef* gene and LTR region in HIV-1 infected partners of HEPS seem to be non-transmitted to the partner, who has repeated unprotected sexual relation for several years. These cases might have low inoculum level or too defective virus to transmit to their partners. The finding of HIV-1 variants with *nef* deletion in HIV-1-infected partners of HEPS provides additional impetus for consideration of the vaccine approach.