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KANTIMA SANGSIRIWUT : DISTRIBUTION OF HIV-1 DNA IN CD4+ T-CELL SUBPOPULATIONS WITH DIFFERENT CHEMOKINE RECEPTORS. THESIS ADVISORS : PRASERT AUEWARAKUL, M.D., Dr Med, RUENGPUNG SUTTHENT, M.D., Ph.D., KOVIT PATTANAPANYASAT, Ph.D. 110 P. ISBN 974-664-581-1.

The major HIV-1 chemokine receptors, CCR5 and CXCR4, have been shown to be differentially expressed in subpopulation of CD4+ T cells. CCR5 is expressed mainly on CD45RO memory, activated T cells, while CXCR4 is expressed mainly on CD45RA naïve T cells. To investigate whether viruses with different cell tropism infect different CD4+ T cell subsets *in vivo,* the distribution of HIV-1 proviral DNA in CCR5+CD4+ and CCR5-CD4+ T cell subsets from 30 infected individuals at different stage of disease was determined by competitive nested PCR method. The V1V2 and V3 regions were determined by PCR-direct sequencing and viral phenotype were predicted from the deduced amino acid sequence.

The HIV-1 proviral DNA load in unsorted CD4+ T cells had a broad range from 85 to 6,734 copies/10^5 CD4+ T cells with a median of 389 copies/10^5 CD4+ T cells. There was an inversed correlation between proviral DNA load and CD4 counts ($r = -0.85$, $p = 0.01$, Spearman correlation coefficient).

In all patients HIV-1 provirus could be detected within CCR5+CD4+ and CCR5-CD4+ T cells. The HIV-1 proviral DNA load in CCR5+CD4+ T cells had a broad range from 201 to 3,921 copies/10^5 CD4+ T cells and HIV-1 proviral DNA load in CCR5-CD4+ T cells had a broad range from 24 to 5,157 copies/10^5 CD4+ T cells. The CCR5+CD4+ T cells had a median of two-fold more provirus than CCR5-CD4+ T cells. There was correlation between the ratio of HIV-DNA load in CCR5+CD4+ T cell / CCR5-CD4+ T cell and CD4 count ($r = 0.76$, $p < 0.01$, Spearman correlation coefficient). When comparing the ratio of HIV-DNA load in CCR5+CD4+ and CCR5-CD4+ T lymphocyte subsets, we found that this ratio in patients with CD4 count < 200 cell/mm^3 were less than those in patients with CD4 count > 200 cell/mm^3($p=0.02$, Mann-Whitney U test).

All V3 sequences were grouped as HIV-1 subtype E. Ten of 28 infected individuals were predicted as SI and 18 were predicted as NSI viruses. When comparing the ratio of amount of proviral DNA in CCR5+CD4+ and CCR5-CD4+ T lymphocyte subsets, we found that the ratio in individuals with predicted SI virus were less than in individuals with predicted NSI virus ($p = 0.07$, Mann-Whitney U test). We concluded that NSI or CCR5-using viruses preferentially infect CCR5+CD4+ T cells, while SI or CXCR4-using viruses preferentially infect CCR5-CD4+ T cells.

However, among those samples with low HIV-DNA in CCR5+CD4+/ CCR5-CD4+ ratio, about only half of them showed SI predicted phenotype (4 out of 9 samples with ratio <1 and 10 out of 20 with ratio < 5). We found some patients who were infected by predicted NSI virus had low level of proviral DNA load ratio and low CD4 count. Infact, the sample with the lowest ratio (K18) had predicted NSI phenotype. It might indicate that, the disease might progress even when the viruses remain to be NSI. The infection of CCR5 negative cells by R5 viruses might be due to the ability of these viruses to use CCR5 at subdetectable concentration.