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学位論文名	Interleukin-4 Upregulates T-Tropic Human Immunodeficiency Virus Type 1 Transcription in Primary CD4$^+$ CD38$^+$ T-Lymphocyte Subset （CD4$^+$ CD38$^+$ Tリンパ球サブセットにおけるIL-4依存性T細胞指向性HIV-1の転写促進）
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論文内容の要旨

Objective

Disease progression after infection with human immunodeficiency virus type 1 (HIV-1) is correlated with virus burden, which seems to be derived from the efficiency of HIV-1 progeny particle production from CD4$^+$ T lymphocytes. The CD4$^+$ T lymphocytes mature into functionally heterogeneous subsets from a common lineage by a process of division, migration, selection, differentiation, and proliferation. Such subsets are classified by differentiation or activation markers. Therefore, several studies have focused on the differences in the susceptibility of the T-cell subsets to HIV-1 infection. The capacity of HIV-1 to infect resting CD4$^+$ T cells and to produce progeny particles may contribute significantly to its pathogenicity in vivo. We previously reported that primary culture of resting CD4$^+$ CD38$^+$ T-cell subset had higher production rate of CXCR4-using X4 HIV-1 than CD4$^+$ CD38$^-$ subset. This work is to search the mechanisms which contribute to the different virus production rate between these two subsets.

Materials and Methods

HIV-1 laboratory strain, CXCR4-using NL4-3, was prepared from the culture medium of 293T cells transfected with pNL4-3. For the preparation of vesicular stomatitis virus glycoprotein VSV-G pseudovirus with NL4-3 backbone containing deletion at the *env* and a luciferase gene in place of *nef*, 293T cells were co-transfected with pNL-ΔEnvΔNef/Luc and the VSV-G expression vector pHIT/G using the Calcium Phosphate Transfection Kit. Isolation of CD4$^+$ T cells fraction from healthy donor-derived peripheral blood mononuclear cells (PBMCs) was performed by depletion of non-CD4$^+$ T cells. CD4$^+$ T-cell population was further separated into CD38$^+$ and CD38$^-$ subsets with MAb to CD38. The CD38$^+$ and CD38$^-$ subsets of the CD4$^+$ T-cell population were pretreated with IL-4 or phytohemagglutinin (PHA) for 3 days, then infected with HIV-1 NL4-3 or VSV-G pseudovirus at the same amounts of virus. Flow cytometry was used to detect the expression of Ki-67 and CD25.

Semiquantitative Alu-PCR was used to compare the integrated DNA level. Northern blotting was performed to compare the virus RNA level. Western blotting was used to detect the phosphorylated Stat 6. Electrophoretic mobility shift assay was used to examine the binding activity of the transcription factors (AP-1 and NF-κB).

Results

The $CD38^+$ and $CD38^-$ subsets were pretreated with IL-4 followed by NL4-3 HIV-1 infection. The result showed that high and low susceptibilities to HIV-1 infection were found in the $CD38^+$ and $CD38^-$ subsets, respectively. In contrast, the same two subsets pretreated with PHA followed by NL4-3 infection showed similar levels of virus production. Flow cytometric analyses revealed no apparent difference in the expression rates of CD25 and Ki-67 between IL-4-treated CD38 subsets. There were no apparent differences between IL-4-treated $CD4^+$ $CD38^+$ and $CD4^+$ $CD38^-$ subsets in the initial steps of HIV-1 replication, such as adsorption, penetration, and reverse transcription. Also, the level of the integrated provirus was similar between both IL-4-treated subsets. In contrast, the viral mRNA level was significantly higher in IL-4-treated $CD38^+$ subset than $CD38^-$ subset. In addition, higher luciferase activity in $CD38^+$ than $CD38^-$ subset was also observed by infection with VSV-G pseudovirus. Interestingly, the NF-κB binding activity was not different between the IL-4-treated subsets, while AP-1 binding activity was much higher in the IL-4-treated $CD38^+$ than $CD38^-$ subset.

Conclusion

Higher susceptibility in the IL-4-treated $CD38^+$ than $CD38^-$ subset to X4 HIV-1 occurred at the transcription step. Also, higher activation of the transcription factor AP-1 was detected in the $CD38^+$ than $CD38^-$ subset after IL-4 treatment. Thus, these results suggest a significance of the AP-1, that was activated by IL-4, on higher X4 HIV-1 production rate of the $CD38^+$ compared with $CD38^-$ subset. Further study is necessary to clarify the mechanism for the AP-1 function on the upregulation of X4 HIV-1 transcription in the $CD38^+$ subset.

論文審査の結果の要旨

Tリンパ球指向性（X4）のヒト免疫不全ウイルス１型（HIV-1）は、長期の無症候性キャリア期を経てAIDS発症に近づくと増加する。また、病態進行とともにCD4＋ T細胞のうち、CD38＋/CD38－比が徐々に上昇する。さらに、当研究室で、CD4＋CD38＋サブセットがIL-4 依存的にX4 HIV-1 に高感受性を示すことが報告されていた。本研究では、このCD4＋CD38＋サブセットがX4 HIV-1 感染に高感受性を示す機序について比較研究を行った。

その結果、ウイルスの転写過程において両サブセット間で顕著な差異が認められた。そこで、HIV-1 転写に関わる宿主転写因子について検討し、AP-1 がIL-4 依存的にCD4＋CD38＋サブセットで、より活性化されることを見出した。

李永剛君の研究内容は、病態の進行とともに増加してくるX4 HIV-1 が、CD4＋ T細胞のCD38サブセットにおいて転写過程で制御を受けていることを示したものである。したがって、本研究は、今後、新たな抗 HIV-1 薬開発に向けて示唆を与えるものであり、学位の授与に値すると考えられる。