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学位授与の要件	学位規則第４条第１項該当 医学系研究科予防環境医学専攻
学位論文名	Human herpesvirus-6 infection induces the reorganization of membrane microdomains in target cells, which are required for virus entry （ヒトヘルペスウイルス６は標的細胞に侵入するために、細胞膜上のマイクロドメインを再構築する）
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論文内容の要旨

〔 Purpose 〕

Human herpesvirus 6 (HHV-6) is a betaherpesvirus. HHV-6 isolates can be classified into at least two variants, variant A (HHV-6A) and variant B (HHV-6B), based on their genetic, antigenic, and growth characteristics. Human CD46 is a cellular receptor for HHV-6A, and an HHV-6A envelope glycoprotein complex, consisting of glycoprotein H (gH), glycoprotein L (gL), glycoprotein Q1 (gQ1), and glycoprotein Q2 (gQ2), serves as the viral ligand for human CD46. Recently, cell-membrane raft microdomains have been shown to be important for successful infection by several viruses. However, their role in the cell-entry process of HHV-6 is unknown. Although viral membrane cholesterol is required for the entry of HHV-6A, whether or not cholesterol is required in the cell membrane remains to be elucidated, and it is unknown if human CD46 and its viral ligand associate in the lipid rafts during HHV-6A entry. Therefore, the purpose of this research is to investigate the role of cellular lipid rafts in HHV-6 entry.

〔 Methods and Results 〕

HSB-2 cells (T cell line) and HHV-6A GS strain were used in this study. HSB-2 cells were treated with various concentrations of methyl-β-cyclodextrin (MβCD) which is a cholesterol-sequestering agent, for 30 min at 37°C, and the pre-treated cells were infected with HHV-6. Sixteen hours post-infection, the expression levels of IE1 protein, an immediate early protein, were determined by Western blot. The cholesterol depletion of HSB-2 had a strong inhibitory effect on the infection that was noticeable even at 1 mM MβCD. In fact, densitometry showed that the level of IE1 in the cells treated with 1 mM MβCD was approximately 50% of that in untreated cells. IE1 was detected faintly in cells treated with 2.5 mM or 3 mM MβCD. The results showed that the cholesterol depletion of HSB-2 had a strong inhibitory effect on the infection.

Next, to examine whether the effect of MβCD was permanent or reversible and to confirm that the effects of MβCD were solely due to cholesterol depletion, exogenous cholesterol was used to replenish the cell surface

of MβCD-treated cells. The addition of 0.1 mM cholesterol to cells treated with 2.5 mM MβCD restored the expression of IE1. However, the IE1 levels were lower than in cells that were not treated with MβCD, indicating that the exogenous cholesterol was able to restore virus entry only partially.

Generally, the initial step of virus entry into a host cell is the attachment of the virus to the cell surface, which is followed by the fusion of the viral envelope with the cell membrane or by the internalization of virus. Therefore, we explored the possibility that MβCD treatment impairs the binding of HHV-6 to the cell surface. HSB-2 cells treated with 2.5 mM MβCD were incubated with HHV-6 for 1 hour at 4°C. Virion binding was analyzed by FACS. The expression level of envelope protein was essentially the same in the MβCD-treated and untreated cells. Therefore, the depletion of cholesterol from the cell surface did not affect HHV-6's binding to the cell.

Next we examined whether the HHV-6A receptor, CD46, was present in the lipid rafts following the initial virus association with the cell membrane or during viral entry. HSB-2 cells were infected with the purified HHV-6 virions. At 1 hour post-infection, the cells were lysed with Triton X-100. The lysates were spun through a sucrose gradient. Interestingly, although CD46 was detected only in the high-density fractions in the uninfected cell lysates, in the HHV-6-infected cell lysates, it was detected in the low-density fractions as well. These results suggested that CD46 was distributed into the detergent-resistant membrane microdomains (DRMs) immediately after HHV-6 attachment.

The requirement for cholesterol for HHV-6 entry suggested that the envelope proteins interacted with the lipid rafts. To test this possibility, we looked for viral envelope glycoproteins in the DRM-containing fractions by Western blot analysis. The gQ1, part of a viral glycoprotein complex that binds CD46, were detected in the lipid raft fractions of HHV-6A-infected cells as well as in the high-density fractions. The results suggest that viral envelope glycoprotein associate with cellular lipid rafts during the entry process.

〔 Conclusions 〕

Intact cholesterol on the cell membrane, as well as the viral membrane, is required for the successful entry of HHV-6. Furthermore, the receptor for HHV-6 is distributed in the lipid rafts during viral entry, indicating that HHV-6 infection induces the re-location of its receptor into the rafts. These observations suggest that lipid rafts in the cellular membrane play an important role in the viral entry process and that HHV-6 may enter target cells via the lipid rafts.

論文審査の結果の要旨

申請者は本論文において、ヒトヘルペスウイルス６（HHV-6）の細胞侵入過程における、宿主細胞膜の脂質ラフトの役割を解析した。脂質ラフトはスフィンゴ脂質とコレステロールに富む細胞膜ミクロドメインで、情報伝達や膜輸送の場としてはたらく一方、細菌やウイルスの細胞内侵入もしくは細胞外放出の場としても近年注目を集めている。HHV-6に関しては、これまでにウイルスエンベロープのラフトが細胞侵入に必須であるとの報告はあったが、細胞膜のラフトの機能を明らかにした報告は本論文が初めてである。

申請者は細胞のコレステロール除去によってウイルス侵入が阻害されることを見出した。この操作による、レセプター分子CD46の発現量やウイルスの細胞表面への吸着量に変化はなく、侵入過程が直接阻害されていることを示唆する結果であった。さらに申請者は、CD46とウイルスリガンド糖タンパクが感染直後にラフトに集積することを発見した。特にレセプターのラフトへの集積は、他のヘルペスウイルスでは見つかっていない現象であり興味深い。本研究により、HHV-6が宿主細胞膜のラフトを介して細胞侵入することが明らかとなった。

未だ不明な点が多いHHV-6の感染機構の一端を解明した本論文は、今後の同ウイルス研究の展開に大きく寄与するものであり、博士（医学）の学位授与に値するものと判断する。