



Title	Isolation and propagation of Dengue virus in Vero and BHK-21 cells expressing human DC-SIGN stably
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論文内容の要旨
Synopsis of Thesis

氏名 Name	Supranee Phanthanawiboon
論文題名 Title	Isolation and propagation of Dengue virus in Vero and BHK-21 cells expressing human DC-SIGN stably (DC-SIGN発現Vero細胞およびBHK-21細胞を用いたデングウイルス分離と増殖)
論文内容の要旨	
〔目的(Purpose)〕	
<p>Dengue is a major public health problem in tropical and subtropical countries. It causes a broad range of symptom from mild to severe. Dengue viruses need to be isolated and propagated from the sample of patients or mosquito to use for research, however; the isolation and propagation system is not fully efficient. DC-SIGN is believed to be a dengue receptor and it was reported to facilitate virus infection. The cell lines using in conventional method has no DC-SIGN expression therefore expression of DC-SIGN on these cells might improve their susceptibility to dengue and also might increase a chance of binding to virus population. This study wants to develop a new method for dengue virus isolation and propagation.</p>	
〔方法ならびに成績(Methods/Results)〕	
<p>Lentivirus system was used to introduce human DC-SIGN gene into Vero and BHK-21 cell. Expression of the DC-SIGN protein on cell membrane was confirmed by IFA (Immunofluorescent assay) and Western blot assay. Vero and BHK-21 expressing DC-SIGN cells were examined viral susceptibility compared to C6/36 and their original cells by using 5 laboratory strains and 13 clinical viruses. The virus titer was determined by focus forming assay using Vero cell. The results showed that DC-SIGN expressed Vero and BHK-20 cells have higher susceptibility to the laboratory strain viruses compared to their original cells. For isolation, C6/36 inoculated with buffy coat from patient samples gave highest isolation rate compare to mammalian cells. After a passage through C6/36, the propagation rate in all cell type are similar and Vero expressing DC-SIGN give the highest virus titer compared to C6/36 and their original cell. Sequence analysis has been done to compare the viruses propagated from C6/36, Vero-DC, BHK-DC and their original cells.</p>	
〔総括(Conclusion)〕	
<p>We have developed Vero and BHK-20 expressing DC-SIGN and these two cell lines show higher susceptibility to propagate the dengue virus laboratory strains and passaged-viruses compared to original cells. Mammalian cells show lower clinical virus isolation rate than mosquito cell. This might due to cross reaction ability of the inhibitory factors from clinical sample among mammalian species. Once passage through the mosquito cell these inhibitory factors were diluted and the number of the virus also increased therefore the passaged-viruses can replicate well in mammalian cells and Vero expressing DC-SIGN showed highest virus replication. These data suggest that Vero-DC and BHK-DC could be useful tools for virus propagation, and that human specimens may contain a factor that interferes with virus growth in mammalian cells.</p>	

論文審査の結果の要旨及び担当者

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論文審査の結果の要旨		
<p>デングウイルス感染症は熱帯、亜熱帯地域で流行している蚊媒介性の熱性疾患であり、重症化した場合死に至ることがある。デングウイルスは、培養細胞でのウイルス分離・產生効率が低く、研究の障害になっている。本研究では高効率のウイルス分離法、増殖法の開発を試み、Phanthanawiboonさんはデングウイルスの既知レセプターであるDC-SIGNを安定に発現する哺乳類由来Vero細胞（Vero-DC細胞）、BHK-21細胞（BHK-DC細胞）を樹立した。樹立細胞では、デングウイルス実験室株は高効率でウイルス產生が起こることが確認できた。また臨床サンプルを用い、13人のタイ人感染者由来血液サンプルを用いてウイルス分離を行ったところ、一度ウイルスをC6/36細胞で分離すると、その後C6/36細胞でウイルス產生させるよりも高いウイルス產生を得られたことを見出した。DC-SIGNは主なレセプターと考えられていたが、デングウイルスはその他のレセプターも使用して患者生体で効率よく感染していると考えられた。これら一連の研究は、デングウイルス研究分野の疑問答える有意義な成果であり、学位の授与に値すると考えられる。</p>		