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Dengue viruses can infect human primary lung epithelia as well as lung carcinoma cells, and can also induce the secretion of IL-6 and RANTES

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Abstract

Dengue viruses (DENV) are herein demonstrated for the first time as being able to infect and replicate in human primary lung epithelium and various lung cancer cell lines. The detection of dengue virus particles and viral negative strand RNA synthesis in the cell, in conjunction with the release of viral progenies in culture supernatants, support the notion that lung cells are susceptible to dengue virus infection. The replication efficiency of DENV in lung cancer cells from high to low is: DEN-2 (dengue virus type-2), DEN-3, DEN-4 and DEN-1. Moreover, the susceptibility of the six lung cancer cell lines to DEN-2 infection is: SW1573 > A549 > H1435; H23; H520; Bes2B. DEN-2 infection significantly increased the expression levels of IL-6 and RANTES in four of the six lung cancer cell lines, which is consistent with the high expression levels of these molecules in DHF/DSS patients. IL-6 expression induced by DEN-2 infection was NF-κB dependent. In summary, our results indicate that lung epithelial cell is a possible target of dengue viruses and IL-6 and RANTES may play pivotal roles in lung related immuno-pathogenesis.

Keywords: Dengue virus; Lung cancer cells; IL-6; RANTES; NF-κB

1. Introduction

Dengue viruses (DENV) are members of the *Flaviviridae* family, and encompass four closely related serotypes: DEN-1, -2, -3 and -4. Patients infected by DENV may display dengue fever (DF), dengue hemorrhage fever (DHF) or dengue shock syndrome (DSS). DF is an acute febrile illness with headache, retro-orbital pain, myalgia, arthralgia, rash, leucopenia, and mild thrombocytopenia. The major pathophysiological change that determines the severity of disease in DHF and differentiates it from DF is plasma leakage (Chaturvedi et al., 1997). The World Health Organization categorizes DHF into four grades, from less severe (grade 1) to severe (grade 4). Grades 3 and 4, in which plasma leakage is so profound that shock occurs, are also referred to as DSS (Chaturvedi et al., 1999). The development of DHF provides warnings of an increased probability of shock. The risk of DHF/DSS is higher in secondary infections with DEN-2 compared with other serotypes (Rico-Hesse et al., 1997).

Viral antigens are found in the alveolar macrophages, pulmonary vascular endothelium, and monocytes inside the blood vessels of the lung (Jessie et al., 2004). It is postulated that alveolar macrophages carrying the DENV antigen have been recruited into the lung. Although the pulmonary and pleural effusions are thought to be caused by vascular endothelium permeability change (Liam et al., 1993), it is not clear whether infiltrated

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macrophages can carry DENV to the lung causing lung cell infection.

IL-6, is responsible for multiple local and systemic alterations during acute inflammatory reaction (Akira et al., 1993). The interleukin species, synthesized by monocytes/macrophages, neutrophils and endothelial cells, is capable of stimulating neutrophil chemotaxis and releasing enzymes, thereby leading to tissue destruction when overproduced (Liu et al., 2003). IL-6 has been implicated in the regulation of aberrant function in inflammatory states and is increased in blood (Ayala et al., 1991; Ertel et al., 1995) and edema fluid (Schutte et al., 1996) after tissue injury. IL-6 plays an important role in the generation of edema during inflammatory processes (Maruo et al., 1992). Addition of IL-6 to uninjured human umbilical vein endothelial cell (HUVEC) monolayers causes reversible alterations in permeability (de Vries et al., 1996) by rearranging actin filaments and thus changing the shape of endothelial cells.

RANTES (regulated upon activation, normal T cell expressed and secreted), a member of the C–C chemokine family (Schall et al., 1988), can be induced by different viruses, such as respiratory syncytial virus (RSV) in bronchial epithelial cells, measles virus in astrocytoma cells, influenza A virus in airway epithelial cells, and dengue virus in endothelial and hepatoma cells (Avirutnan et al., 1998; Huang et al., 2000; Lin et al., 2000; Matsukura et al., 1998; Thomas et al., 1998). RANTES probably plays a major role in recruiting leukocytes to the areas of tissue damage caused by RSV replication (Appay and Rowland-Jones, 2001). Several reports have shown that epithelial cells are a major source of RANTES production in the lung, and virus-induced RANTES expression could be a major event in the pathogenesis of viral infection (Alam et al., 1996; Gonzalo et al., 1998). Consistent with this role, inhibition of RANTES production appears to produce a beneficial effect in controlling bronchial asthma induced by influenza virus infection (Asai et al., 2001).

The NF-κB and p38-MAPK pathways, are involved in the regulation of cytokines at the transcriptional and biosynthetic levels (Haddad, 2002). In the NF-κB pathway, phosphorylation of an inhibitory-κB (IκB) protein controls the translocation of NF-κB complex from cytosol to nucleus in activating gene expression. NF-κB is a key transcription factor for various pro-inflammatory molecules, such as chemokines, cytokines, and adhesion molecules (Liu et al., 2003). IL-6, RANTES and NF-κB have been reported to play pivotal roles in various cells after dengue virus infection. However, their roles in dengue virus infected lung cells have not been reported.

In this study, we utilized an *in vitro* culture system to reveal that DENV could infect primary lung epithelial cells as well as lung carcinoma cells. We also explored the lung-related pathogenesis related to DENV infection.

2. Materials and methods

2.1. Viruses and cell culture

DENV serotypes 1–4 (DEN-1: strain 766733A, DEN-2: strain PL046, DEN-3: strain 739079A, and DEN-4: strain H-241) were maintained in C6/36 cells. The titer was evaluated in BHK-21 cells by plaque assay (Anderson et al., 1997). DEN-2 heated at 56 °C for 30 min was used as inactivated dengue virus (iDV) (Avirutnan et al., 1998; Marianneau et al., 1999). For virus infection experiments, cells were adsorbed with DENVs at multiplicity of infection (m.o.i.) of 5–100 at 37 °C for 2 h. The cells were then washed three times with phosphate buffered saline (PBS, pH 7.4) and incubated at 37 °C in the normal medium.

A549 cells (type II human lung alveolar epithelial cell carcinoma) were maintained in Ham's F12K medium (GIBCO-BRL, Carlsbad, CA, USA) with 10% fetal calf serum (FCS) (Biological Industries, Kibbutz Beit Haemek, ISRAEL). SW1573 cells (alveolar epithelial cell carcinoma) were maintained in Leibovitz's L-15 medium (GIBCO) with 10% FCS. H23, H1435 (adenocarcinoma; non-small cell lung carcinoma), H520 (squamous cell carcinoma) and Bes2B (bronchus; epithelial; virus transformed; normal) cells were maintained in RPMI 1640 medium (GIBCO) with 10% FCS. They were obtained from the American Tissue Type Collection (ATCC, Rockville, MD, USA) and cultured at 37 °C in a 5% CO_2 incubator. The primary lung cells from human tissue (lung carcinoma patient and collected by Dr. Wu-Wei Lai, National Cheng Kung University Hospital) were treated with 0.1% trypsin (1:250, Sigma, St. Louis, MO, USA) for 1 h at 37 °C, and cultured in DMEM containing 30% FCS.

2.2. Immunohistochemical staining

Human lung epithelial cancer cells and lung primary cells were cultured on cover slips, and fixed with 3.7% formaldehyde solution at 37 °C for 15 min. The cells were rinsed with PBS, and then covered with blocking buffer (1% BSA in PBS) at 37 °C for 60 min. The primary monoclonal antibody (anti-DEN E or NS1 protein obtained from Dr. Huan-Yao Lei, National Cheng Kung University) was distributed on each cover-slips and incubated at 4 °C over-night. The cover-slips were washed three times with PBS. Enough link antibody solution (DAKO LSAB2 System; Carpinteria, CA, USA) was added to cover the surface of each cover-slip (150–200 ul), and incubated for 10 min at room temperature (RT). Streptavidin–HRP solution (DAKO LSAB2 System) was added to cover the surface of each cover-slip after PBS washed three times, and incubated for 10 min at RT. The cover-slip was washed three times with PBS. AEC chromogen substrate was then added until the red color appeared.

2.3. Immunofluorescent staining

The cell preparation and the staining protocol are the same as the above procedure. The primary antibody (mouse IgG-conjugated goat anti-human cytokeratin and human serum) was diluted to 1.0 μl/ml in blocking buffer, and was distributed on each cover-slips and incubated at 4 °C over-night. The secondary antibody (rhodamine-conjugated goat anti-human IgG and FITC-conjugated goat anti-mouse IgG; Invitrogen, Carlsbad, CA, USA) was diluted and added in the appropriate concentration to the blocking buffer, and incubated with the