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Pathogenesis of Coxsackievirus B

Heuiran Lee
Dept. of Microbiology, College of Medicine University of Ulsan

Abstract

The aim of this study is to elucidate the cytopathic effect on various susceptible cells of coxsackievirus B (CVB) infection. Coxsackievirus, along with poliovirus (PV), belongs to the enterovirus genus in the family *Picornaviridae*, which encloses a single-stranded polyadenylated RNA as a genome. Based on their pathogenecity in newborn mice, coxsackievirus is subclassified into coxsackievirus A and CVB of serotype CVB1 to 6. CVB often causes a wide spectrum of human illness varying from mild symptoms of common cold or aseptic meningitis to more severe diseases including encephalitis, and myocarditis.

Previous studies have implied that CVB infections in susceptible cells cause productive virus replication, cell death, and lysis of the infected cells, facilitating the dissemination of viral progeny. Moreover, it has become apparent that apoptotic cell death plays an important role in the pathogenesis of human viral diseases. Similar to other viruses, several studies have demonstrated that apoptotic characteristics were detected in several types of cells infected with certain CVB serotypes. In cultured rat cardiomyocyte, apoptotic cell death was noticed within 36 h following CVB3 infection by both deoxy-nucleotide transferase directed d-UTP nick and end labeling (TUNEL) staining and transmission electron microscopy (TEM). CVB3 also induces degenerative morphological changes in infected HeLa cells accompanied with caspase 3 activation. Despite these facts, neither the nature of cytopathogenesis in various susceptible cells nor the major cause of preferential association by different serotypes of CVB remained poorly understood.

In the present study, we therefore investigated the characteristics of the cytopathic effects following infection with all 6 different CVB serotypes in both Vero cells and primary neural cells. All the CVB serotypes were also obtained from ATCC (CVB1, VR-687; CVB2, VR-29; CVB3, VR-30; CVB4, VR-184; CVB5, VR-1036; CVB6, VR-1037). The viruses were propagated and titered by plaque assay in Vero cells. postinfection (p.i.). The Vero cell has been widely utilized as a permissive cell to grow both prototype CVBs and their clinical isolates Morphological and biochemical alterations of the infected cells were then examined by light microscopy (LM), MTT or LDH assay, Hoechst staining, oligonucleosomal DNA degradation analysis, and transmission electron microscopy (TEM). Virus multiplication was also determined by one-step growth curve at various times p.i.

The data indicated that the infected permissive Vero cells, regardless of virus serotypes, experienced similar degrees of CPEs within 24 h p.i. Using both Hoechst 33342 staining and TEM,

we consistently observed morphological properties of apoptosis, heavily condensed nuclei, and subsequent chromatin condensation into the periphery of the nuclei within 12 h p.i. Moreover, we noticed typical oligonucleosomal DNA fragmentation, while productive CVB multiplication was accomplished within 6 h p.i. prior to an apoptotic signal. Caspase inhibitor Z-VAD.fmk significantly prohibited nuclear change by apoptosis with no influence on virus production and cell death, demonstrating that all the CVBs induced more than one type of pathological effect including apoptotic alteration in permissive Vero cells.

Contrary to Vero cells, the infected mouse primary cortical neural cells experienced various degrees of CPEs within 24 h p.i. Both morphological alteration under LM and LDH assay consistantly indicated that CVB4 and CVB5 induced the most severe cytopathic effect, while CVB2 and CVB6 did the least. Yet, cytopathic effect induced by CVB infection seemed to be due to induction of apoptotic cell death, demonstrated by both LM and Hoechst 33342 staining. Similar to the infected Vero cells, Z-VAD.fmk only prohibited apoptotic nuclear change with no influence on virus production and cell death. This finding may indicate that the mechanism for cytotoxicity in infected Vero cells and primary neural cells is similar. However, this study also suggested that the severity of cytotoxicity by different CVB serotyps only varied in primary neural cell. Currently, we are attempting to elucidate the cause of variation in cytopathic effects in primary neural cells by different CVB serotypes.

References

1. Rueckert RR: Picornaviridae: The viruses and their replication.; in B. Fields DKaPH (ed): Fields Virology. New York, Raven, 1996, pp 609-654.
2. Muir P: Enteroviruses and heart disease. Br J Biomed Sci 1993;50: 258-71.
3. Nigrovic LE: What's new with enteroviral infections? Curr Opin Pediatr 2001;13: 89-94.
4. Melnick JL: Enteroviruses: Polioviruses, Coxsackieviruses, Echoviruses and newer enteroviruses.; in B. Fields DKaPH (ed): Fields Virology. Philadelphia, Lipponcott-Raven, 1996, pp 655-712.
5. White E: Life, death, and the pursuit of apoptosis. Genes Dev 1996;10: 1-15.
   Arends MJ, Wyllie AH: Apoptosis: mechanisms and roles in pathology. Int Rev Exp Pathol 1991;32: 223-54.
6. Li BY, Qiao GF, Zhou H, Li WH, Huang ZG, Zhou LW: Cytosolic-Ca2+ and coxsackievirus B3-induced apoptosis in cultured cardiomyocytes of rats. Zhongguo Yao Li Xue Bao 1999;20: 395-9.
7. Carthy CM, Granville DJ, Watson KA, Anderson DR, Wilson JE, Yang D, Hunt DW, McManus BM: Caspase activation and specific cleavage of substrates after coxsackievirus B3-induced cytopathic effect in HeLa cells. J Virol 1998;72: 7669-75.
8. Joo CH, Kim YK, Lee H, Hong H, Yoon SY, Kim D: Coxsackievirus B4-induced neuronal apoptosis in rat cortical cultures. Neurosci Lett 2002;326: 175-8.
9. Agol VI, Belov GA, Bienz K, Egger D, Kolesnikova MS, Romanova LI, Sladkova LV, Tolskaya EA: Competing death programs in poliovirus-infected cells: commitment switch in the middle of the infectious cycle. J Virol 2000;74: 5534-41.