

Induction of Apoptosis in MRC-5, Diploid Human Fetal Lung Cells after Infection with Human Coronavirus OC43

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1. INTRODUCTION

Human coronaviruses (HCoV) cause upper respiratory tract infections manifested by symptoms of the common cold including rhinitis, tussis, fever, headache and myalgia (Hruskova *et al* 1990). HCoV respiratory illness is primarily virus-mediated; immunologic events have not been postulated to play a role (Makela *et al* 1998). Evidence showing the presence of viral genome in spinal fluid and brain suggests that HCoV may play an etiologic role in multiple sclerosis (Stewart *et al* 1992, Cristallo *et al* 1997). However, the pathogenesis of HCoV infections is poorly understood. In human embryonic tracheal organ cultures, HCoV causes a slow patchy destruction of the ciliated epithelial cells and in respiratory epithelial tissue cultures, the cytopathic effect is subtle, evident only by vacuolization and spindling.

Since apoptosis is a mechanism of cell death for other coronaviruses such as transmissible gastroenteritis virus (TGEV) (Eleouet *et al* 1998) and mouse hepatitis virus (MHV) (An *et al* 1999), we examined infected MRC-5 cells with HCoV and examined the cells for changes associated with programmed cell death. DNA fragmentation and formation of apoptotic bodies was observed in cells infected with HCoV-OC43.

2. MATERIALS AND METHODS

2.1 Viruses and Cells

HCoV-229E and HCoV-OC43 strains that had been repeatedly passaged in M-7 and MRC-5, human lung fetal cells were used in this study.

2.2 Assessment of DNA Degradation

HCoV-OC43-infected and uninfected MRC-5 cells (2×10^6 cells) were harvested after six days of exposure to virus. DNA was isolated with DNAzol (Molecular Research Center, Cincinnati, OH), incubated with RNase A ($1 \mu\text{g/ml}$) at 50°C for 1 h, and then loaded onto a 1.2% agarose gel containing ethidium bromide. After electrophoresis, the gel was photographed and examined for the presence of DNA laddering (Jan and Griffin 1999).

2.3 Visualization of Apoptotic Nuclei by Ethidium Bromide Staining

To detect DNA condensation and fragmentation, the cells were fixed in cold acetone, stained for 10 minutes with $100 \mu\text{g/ml}$ ethidium bromide in PBS and washed twice in PBS. Microscopic visualization of apoptotic nuclei was performed by light microscopy using epiillumination and a filter for fluorescein.

2.4 Immunofluorescence

After fixing the cells in cold acetone, viral antigen was detected by indirect immunofluorescent staining using monoclonal antibody 4B6.1 to HCoV-OC43 nucleocapsid as the primary antibody followed by fluorescein-conjugated goat anti-mouse immunoglobulin (Vector, Burlingame CA).

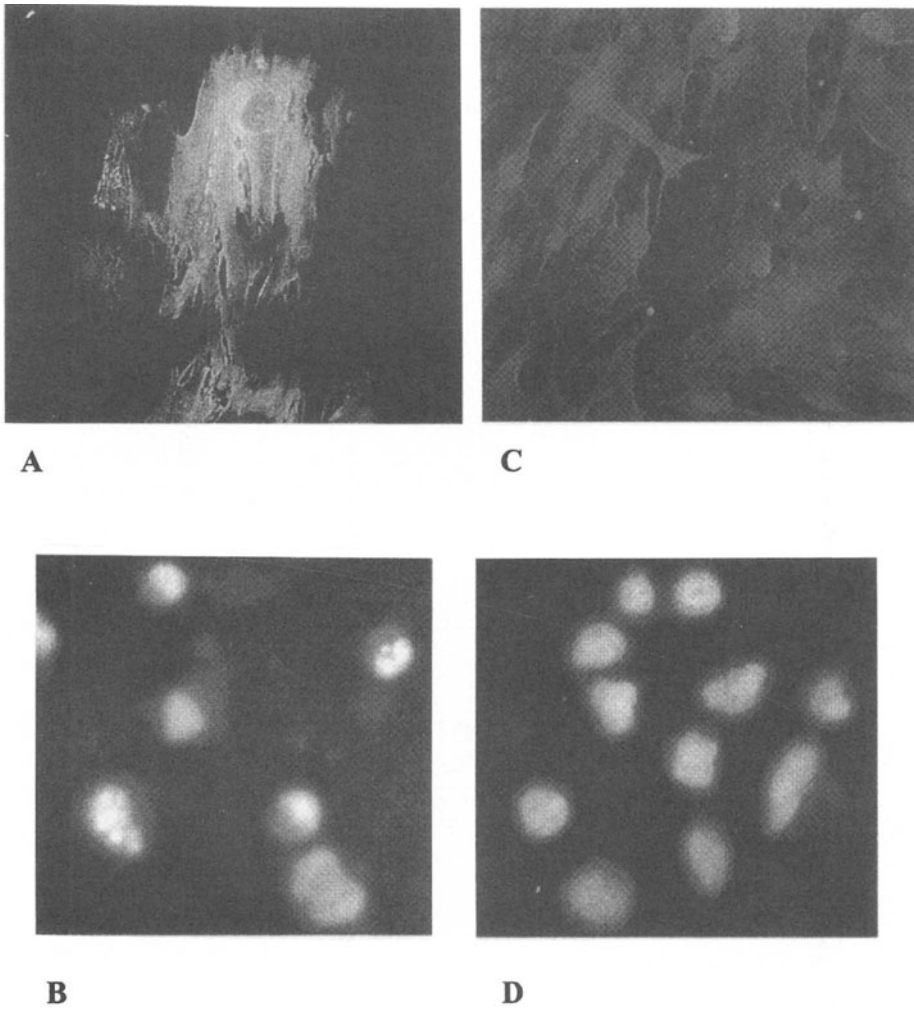


Figure 1. Viral antigen expression and induction of apoptosis in MRC-5 cells infected with HCoV-OC43 at a moi of 1 and incubated at 33°C. Panels A and C: Immunofluorescent staining for viral nucleocapsid antigen with mAb 4B6.1. Panels B and D: Ethidium bromide staining for DNA fragmentation. Panels A and B are infected cells. Panels C and D are mock-infected cells.

3. RESULTS

In order to assess the cytologic changes in MRC-5 cells after infection by HCoV-OC43, we exposed semi-confluent monolayer of cells to virus at a moi of 1 and incubated the cells at 33°C. Three days after infection, swollen, rounded, granular cells were observed. These cells showed immunofluorescent staining for viral nucleocapsid (Fig1 A). By six days post-infection, many cells became detached and those cells remaining were further enlarged and granular. The adherent cells were fixed in acetone and the nuclear DNA was stained with ethidium bromide. We observed DNA condensation and formation of apoptotic bodies in the nucleus (Fig 1B). Further confirmation of DNA fragmentation was obtained by extracting the DNA from a pool of detached and adherent cells infected for six days and assessing for DNA ladder formation after gel electrophoresis. Fragmentation was present in DNA from HCoV-OC43-infected cells, but not in mock-infected cells (Fig.2). Interestingly, DNA fragmentation was observed at six days post-infection indicating that the onset of apoptosis was two to three days earlier, coinciding with the time of maximum viral yield. The yield of infectious HCoV-OC43 was greater in the cell-associated than in the cell-free virus fraction suggesting that accumulation of virus or viral protein(s) caused induction of apoptosis. In HCoV-229E infected MRC-5 cells maximum virus yield was fivefold less and was higher in the cell-free than in the cell-associated fraction. The viral titer reached its peak earlier in HCoV-229E than in HCoV-OC43 infection and the nucleus did not appear to undergo apoptotic condensation.

Table 1. Characteristics of HCoV-OC43-infected MRC-5cells

Property	Characteristic
Cytopathic effect	Granular degeneration, enlargement
Viral titer	2.8×10^6 pfu/ml
Maturation and release	More cell associated
DNA fragmentation	yes
Cell death	apoptosis

4. DISCUSSION

We found evidence of apoptotic death in MRC-5 cells six days after infection with HCoV-OC43, in cells that contained large amounts of intracellular virus. The events that triggered apoptosis have yet to be determined. In MHV-induced apoptosis, the coronavirus E protein alone was responsible (An *et al* 1999). The E protein of MHV, found on gene5, is expressed from an internal ribosome entry structure (IRES) which could

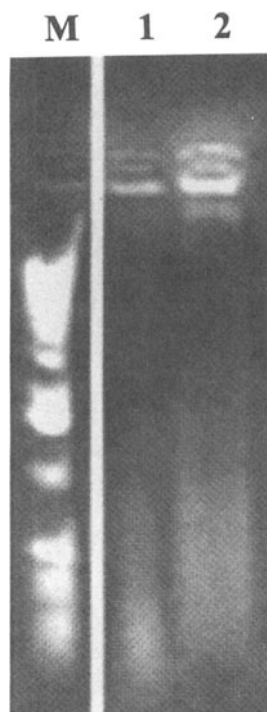


Figure 2. Gel electrophoresis of DNA for assessment of fragmentation in MRC-5 cells. Lane M: 1kb markers, lane 1: mock, lane 2: infected with HCoV-OC43.

account for its accumulation leading to induction of apoptosis (Jendrach *et al* 1999). Induction of apoptosis by HCoV-OC43 may play a role in evasion of immune recognition and suppression of host-mediated responses, events that favor establishment of persistent infection. Other mechanisms favoring persistence include protection of the infected cell from complement-mediated lysis by regulators of complement activation and antigenic variation of the virus (Varsano *et al* 1995, Collins *et al* 1986, Arbour *et al* 1999). In comparison, HCoV-229E infection in MRC-5 cells proceeds more rapidly without programmed cell death. HCoV-229E infected cells may be more sensitive to anti-apoptotic BCL-2 proteins (Bonati *et al* 1996). Interestingly in a study of primary infections showing a seroconversion to the virus, HCoV-229E acute respiratory disease included conjunctivitis and lymphadenitis, symptoms that were not associated with HCoV-OC43 illness (Hruskova *et al* 1990). Further examination of HCoV pathogenesis is important in the management of childhood asthma and older people with chronic illnesses.

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