Susceptibility of Chicken Monocytic Cell Lines to Infectious Bursal Disease Virus

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(Received 10 September 1991/Accepted 21 January 1992)

KEY WORDS: chicken monocyte/macrophage, IBDV virus, susceptibility.

Infectious bursal disease (IBD) is an acute viral disease of young chickens, characterized by necrosis of lymphoid follicles in the bursa of Fabricius [5, 10]. IBDV, the etiologic agent of IBD, is a member of the Birnaviridae family. The major target cells of IBDV are B lymphocytes. Replication of IBDV in chicken B-lymphoid cell lines has been reported [6, 17]. It has also been demonstrated that IgM-bearing B lymphocytes are highly susceptible to IBDV [6, 13]. On the other hand, the presence of IBDV in monocytes and macrophages has been demonstrated after the in vivo [10] and in vitro [1, 12] infections. However, the replicating ability of IBDV in the cells of monocyte/macrophage lineage is not well known. The present report describes the in vitro susceptibility of two chicken monocytic cell lines to FK78 strain of IBDV.

IN24 cells, established from a spontaneous myelocytic leukemia in a chicken [7] were cultured in RPMI1640 medium, supplemented with 10% fetal bovine serum and 10% tryptose phosphate broth at 37°C. Both IN24 and LSCC-NP1 cells were adherent. IN24 cells, which were a promonocytic cell line, possessed an immature cellular character compared with LSCC-NP1 cells [8]. Chicken embryo fibroblast (CEF) cultures were prepared from 9- to 11-day-old embryos of specific-pathogen-free chicken eggs. CEFs were cultured in medium 199 containing 10% fetal bovine serum and 5% tryptose phosphate broth. The FK78 strain of IBDV [16], which exhibited pronounced CPE of virus in CEFs was kindly provided by Dr. Takase (The Chemo-Sero-Therapeutic Research Institute). The virus was grown in CEF cultures until CPE became prominent. The culture medium was harvested and stored at −70°C. IN24 and LSCC-NP1 cells were inoculated with 0.1 ml of viral sample. After adsorption for 60 min, the cell monolayers were washed twice with phosphate-buffered saline and incubated at 37°C in medium, supplemented with 2% fetal bovine serum. At various intervals, the culture medium and cell monolayer in the initial passage of the virus were harvested separately, stored at −70°C and assayed for extracellular and cell-associated viruses. Infectivity titration of the virus was performed in CEFs. The fifty-percent tissue-culture infective dose (TCID50) was calculated. IBDV antigen was demonstrated by the avidin-biotin-peroxidase method [2], using a specific IBDV-antisera prepared in rabbits. Cell suspension was smeared on a coverslip, air-dried for 2 min and fixed with acetone for 10 min. Cell smears were incubated with IBDV-antisera diluted 1:100 at room temperature for 30 min. Immunostaining was performed using an avidin-biotin universal kit (BioGenex Lab., U.S.A.). For electron microscopy, the cells inoculated with the virus were centrifuged to form a pellet. The pellets were fixed in 2.5% glutaraldehyde and 2% osmium tetroxide, dehydrated in ethanol and embedded in epoxy resin. Ultra-thin sections were stained with uranyl acetate and lead citrate.

The CPE of FK78 strain was apparent in both IN24 and LSCC-NP1 cells in the initial passage of the virus. Affected cells showed cell rounding and gradually detached from the substrate (Fig. 1). The CPE was visible in IN24 cells at 30 hr, but indistinct in LSCC-NP1 cells until 48 hr postinoculation (PI). By the avidin-biotin-peroxidase method, about 40% of IN24 cells and 15% of LSCC-NP1 cells reacted positively with IBDV antiserum at 48 hr PI. IN24 cells showed granular reaction product diffusely distributed in their cytoplasm. In the most of

Fig. 1. The CPE of the FK78 strain in IN24 cells at 30 hr PI. Cell rounding and detachment from the substrate are distinct, × 100.
LSCC-NP1 cells, the granular reaction product was restricted to a small cytoplasmic area. Electron microscopically, large clusters of virus particles showing different electron densities and crystalline arrangement were observed in the cytoplasm of IN24 cells (Fig. 2). The cytoplasmic vesicles containing scattered single virus particles were frequently encountered (Fig. 3). No crystalline arrangement of virus particles was detected in LSCC-NP1 cells at 48 hr PI.

IN24 cells were highly susceptible to the FK78 strain in the initial passage of the virus. Extracellular and cell-associated virus titers in IN24 cells steadily rose and reached $10^{7.0}$ and $10^{5.8}$ TCID$_{50}$/0.1 ml at 72 hr PI, respectively (Fig. 4). The titers of extracellular virus exceeded those of cell-associated virus. By comparison, the titers of extracellular virus in LSCC-NP1 cells slightly increased and remained low, $10^{6.8}$ TCID$_{50}$/0.1 ml at 72 hr PI. The titers of cell-associated virus in LSCC-NP1 cells also remained low, but were equivalent to those of extracellular virus throughout the 72 hr observation period. At 48 hr PI, the titer of cell-associated virus, $10^{6.5}$ slightly exceeded the extracellular virus titer, $10^{6.3}$ TCID$_{50}$/0.1 ml.

Two chicken monocytic cell lines, IN24 and LSCC-NP1, were susceptible to FK78 strain of IBDV in vitro. In IN24 cells, the virus rapidly propagated and evidently produced the CPE. IN24 cells showed high susceptibility to the virus in the initial passage of the virus. By contrast, extracellular virus titers in LSCC-NP1 cells slightly increased and remained low throughout the observation period. Previous reports showed that IgM-bearing cells in B-lymphocytes were major target cells for in vitro IBDV infection [6, 13]. On the other hand, it has been...
demonstrated that IBDV can not sufficiently replicate in lymphoblasts and small lymphocytes as well as in bursal cells [1]. Morphologically and functionally, IN24 cells possessed an immature cellular character compared to LSCC-NP1 cells [8]. The investigation for difference of susceptibility to IBDV in IN24 and LSCC-NP1 cells is further required.

The titers of extracellular virus in IN24 cells exceeded those of cell-associated virus. Generally, the titers of extracellular virus were higher than those of cell-associated virus after the in vitro IBDV infection [4, 9, 11, 14, 17]. Cell-associated virus titers in LSCC-NP1 cells were low in contrast to those in IN24 cells, but were equivalent to extracellular virus titers throughout the 72 hr observation period. The virus in LSCC-NP1 cells may not be released readily into the culture medium.

It has been reported that not only the humoral immunity is suppressed in IBDV-infected chicken, but also the cell-associated responses are suppressed [3, 5, 15]. The mechanism for the suppression of cell-mediated responses is not well known. The IBDV infection is suspected to cause functional impairment of monocyte/macrophage. Both IN24 and LSCC-NP1 cells are useful for the in vitro study of IBDV infection.

REFERENCES