Production of Newcastle Disease vaccine using continuous mammalian cells

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Abstract

Specific pathogen free (SPF) eggs have been used to produce live vaccines. however, their application causes many problems such as cost, space and waste disposal. The substitution of mammalian cells for SPF eggs offers a desirable system of vaccine production. In this study, mammalian cells were tested for the infection of Newcastle disease virus (NDV). As a result, DF-1 and MDBK cells showed high virus productivity compared to the other mammalian cells. For the highest productivity of NDV, the optimal multiplicity of infection (M.O.I.) in DF-1 or MDBK cells was determined to be 0.2 or 0.5 M.O.I., respectively.

Introduction

Newcastle disease is a fatal disease spreaded all over the world except the south pole region. It damages the poultry industry seriously. NDV is a single and negative-stranded RNA virus belonging to the family *Paramyxoviridae*. This virus family is divided into two subfamilies, *Paramyxovirinae* and *Pneumovirinae*. This virus has a wide range of host with most orders of birds reported to have been infected by NDV (1, 2, 4). Two envelope glycoproteins, fusion (F) protein and hemagglutinin-neuraminidase (HN) protein play important roles in the initiation of infection (3). HN mediates the binding of the virus to cell surface molecules containing sialic acid, and it also displays neuraminidase activity that probably prevent the aggregation of progeny virus. Both sialidase and hemagglutination activities are affected by the viral membrane composition (4). SPF eggs have been used in the production of NDV-vaccine. However wastes from eggs have caused serious environmental pollution. If NDV vaccine is produced by mammalian cell culture, it can be produced in smaller space,

with lower cost and less labor than that by SPF eggs. In this study, various mammalian cells including DF-1, MDBK, Vero, LLC-PK 1, DT-40, MDCK, BHK-21 and Primary chick fibroblast cell (CFC) were examined to screen suitable cell lines for producing NDV-vaccine. Virus-related intranuclear inclusions seemed to be uncommon in members of *Paramyxoviridae*. The significance of the nucleus for replication of members of *Paramyxovirinae* is not clear yet, since replication and transcription occur independently of nuclear functions (3). So all experiments were performed at the same condition to minimize a variation.

Materials and Method

Eight major cell lines used in experiments screening producers of NDV-vaccine : DF-1 (chicken fibroblast cells), MDBK (bovine kidney cells), Vero (American green monkey kidney cells), LLC-PK 1 (swine kideny cells), DT-40 (chicken lymphoma cells), MDCK (canine kidney cells), BHK-21 (baby hamster kidney cells), COS-7 (SV40 transformed American green monkey kidney cells) (ATCC, U.S.A.). Primay CFC were tested to screen proper cell line.

The cells were routinely cultured in Minimum Essential Medium (MEM) (Gibco, U.S.A.) supplemented with 5% (v/v) fetal bovine serum (FBS) (Hyclone, U.S.A.) and 5% (v/v) tryptose phosphate broth (TPB) (Sigma, USA). Dr. Y. H. Jeong, Kang-Won National University, Chuncheon, Korea, kindly provided the NDV.

Plaque assay was performed using MDBK. After 1 hr adsorption of virus, the inoculum was removed and the testing cells were overlaid with MEM containing 0.5% agar (Seakem, U.S.A.). After incubation at 37℃ for 3 days in a 5% CO_2 atmosphere, agar-overlays are removed and cells were dyed with 1% crystal violet, followed by plaque count.

For preparing primary CFC, SPF eggs (Namduk, Korea) were incubated for 10 days. Chopped 10-day embryo was trypsinized for 10 min. After washing primary CFC with PBS, it was cultured at 37℃ under atmosphere containing 5% CO_2 atmosphere and 95% humid air and subcultured every 4 days.

Results and Discussion

Screening of NDV-infecting mammalian cells

Generally, various veterinary vaccine has been produced in many cell lines from kidney. For NDV-vaccine, however, it has been produced mainly in SPF

eggs because of avian specificity of NDV. In this study, various cell lines from kidney and chick were tested for producing NDV-vaccine (Figure 1).

As a result, MDBK and DF-1 cells showed considerably high productivity of NDV-vaccine. The vaccine productivity in DF-1 and MDBK cells were about 4- and 5- fold higher than that in Vero cells. Compared MDBK to DF-1 virus productivity, plaque forming unit (PFU) of MDBK was 3.8×10^7 PFU/mL and that of DF-1 was 2.6×10^7 PFU/mL. Consequently, the value of MDBK was 1.5-fold higher than that of DF-1. In two cell lines derived from chicken, DF-1 and DT-40, the titer in DF-1 cells was 70-fold higher than that in DT-40 cells. DT-40 showed 3.8×10^5 PFU/mL. The above results indicate that their virus productivities in various mammalian cell lines are different even in the same species. In addition, fibroblast cells have higher virus productivity than lymphoblast.

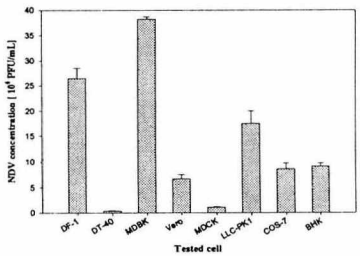


Fig. 1) ND Virus productivity of various cell lines

To determine the NDV productivity in primary CFC, it was infected at different passages. It was done for 5 passage (Figure 2). As a result, whether PFU value was different about 3-fold. but it was not clear virus productivity was affected by cell passage at all.

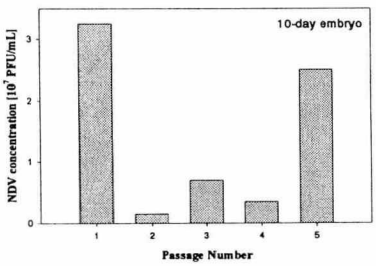
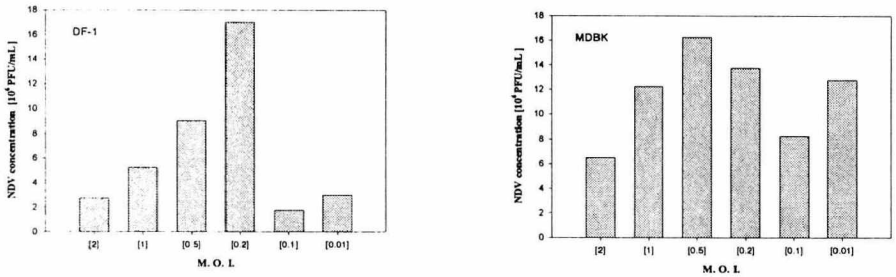


Fig. 2) Productivity of NDV in primary CFC during 5 passages of culture time.

Optimum M.O.I. for producing NDV

To obtain the optimal M.O.I., the experiment was done with DF-1 and MDBK cells. For DF-1 cells, they showed the highest productivity of NDV at 0.2 M.O.I. (figure 3a). While NDV concentration of MDBK cells was maximumat 0.5 M.O.I. (figure 3b). There are two known ways that NDV infects host cells. One is direct infection by HN and F protein on virion, and the other is endocytosis by host cells. However, excess virus can inhibit fusion - competition effect. Since mammalian cells have unique physiological composition, their maximum NDV concentration was obtained at unique M.O.I..



(a) (b)

Fig 3) Effect of M.O.I. on NDV productivity in DF-1 (a) and MDBK (b) cells

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