An Electron Microscopic Study of MDBK Cells Persistently Infected With Newcastle Disease Virus

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With 6 Figures

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Summary

Ultrastructural examination of a line of MDBK cells persistently infected with Newcastle disease virus (MDBKpi cells) revealed the presence of cytoplasmic aggregates of both smooth and granular nucleo capsids. Only granular nucleo capsids aligned under modified areas of plasma membrane and were incorporated into virus particles. On the grounds of morphogenesis, there was no apparent explanation for the persistent, non-cytocidal nature of the infection.

Both nuclear and cytoplasmic aggregates of smooth nucleo capsids were present in MDBKpi cells which had been held without subculture for between 40 and 130 days (aged MDBKpi cells). Modified areas of plasma membrane with associated alignment of nucleo capsids were not present in aged MDBKpi cells, and neither budding nor released virus particles were observed, indicating a block in virus maturation.

It is suggested that the granular material coating granular nucleo capsids allows them to interact with modified areas of plasma membrane, thereby inducing virus budding. A deficiency of this material, as apparently occurs in aged MDBKpi cells, would therefore cause a block in virus maturation. The nature of this granular material is discussed, and we suggest that it consists of M protein.

Introduction

The morphogenesis of a number of paramyxoviruses in productive infections has been described, including Newcastle disease virus (6, 10, 13, 25), Sendai virus (1), parainfluenza virus type 2 (3, 12), parainfluenza virus type 3 (14), mumps virus (9), canine distemper virus (4, 5), and measles and subacute sclerosing panencephalitis (SSPE) viruses (7, 8, 16, 19). However, with the exception of measles and SSPE viruses (8, 20, 21), there are few accounts of electron microscopic studies on long term and persistent paramyxovirus infections, in spite of the
relative ease with which these viruses establish persistent infections in cell cultures. In this paper, we present the results of an ultrastructural study on a bovine kidney cell line persistently infected with Newcastle disease virus (NDV).

**Materials and Methods**

**Cell Cultures**

A line of Madin-Darby bovine kidney cells persistently infected with an unknown strain of NDV was used. The properties of this cell line, which will be referred to as MDBKpi, have been described previously (11).

MDBKpi cells were propagated in 4 oz bottles in Dulbecco's modified Eagle's medium supplemented with 1 per cent 200 mm α-glutamine, and containing 10 per cent heat-inactivated calf serum. The concentration of calf serum was decreased to 3 per cent in maintenance medium. Normally the cells were subcultured weekly, as described previously (11). However, some cultures, referred to as aged cultures, were refed with maintenance medium and held without being subcultured for between 40 and 130 days.

**Electron Microscopy**

Following removal of the medium, cell cultures were fixed *in situ* with ice-cold 3 per cent glutaraldehyde in 0.01 M phosphate buffer, pH 7.2. The cells were then scraped off the glass and left in fresh fixative for 6 hours at 0°C. After several washes in phosphate buffer, post-fixation was carried out in 1 per cent osmium tetroxide in phosphate buffer. Dehydration was performed using a graded series of alcohols, and the cells were embedded in Araldite. Ultrathin sections were stained with uranyl acetate and lead citrate.

**Immunofluorescence**

Coverslip cultures of MDBKpi cells were washed twice in phosphate-buffered saline (PBS) and fixed in ice-cold acetone for 10 minutes. The indirect staining method was employed, using NDV antiserum prepared in rabbits (11) and a fluorescein-labelled antiserum to rabbit globulin prepared in sheep (Grand Island Biological Company, Santa Clara, California). Coverslip cultures were stained for 40 minutes at 37°C in a moist chamber, and washed with PBS for 20 minutes after each staining. Coverslips were mounted in buffered glycerol and examined using ultra-violet illumination. As we were unable to maintain cells long enough on coverslips to establish aged coverslip cultures, the distribution of viral antigen in aged cultures was assessed by staining 24—or 48—hour coverslip cultures obtained by trypsinisation of aged cultures which had previously been maintained in 4 oz bottles.

**Results**

**General Considerations**

The properties of MDBKpi cells have been described in an earlier communication (11). From 1962 to 1971, it was not possible to detect infectious virus in MDBKpi cultures. However, since 1971, virus infectious for chick embryo fibroblasts has been consistently released from MDBKpi cells, but infectivity titres of this virus in the medium removed from MDBKpi cultures were low, usually between $10^1$ and $10^2$ TCID$_{50}$ per ml. As large numbers of virus particles were released from MDBKpi cells (see below), it is probable that the majority of these particles were defective in infectivity. Immunofluorescent staining of coverslip cultures of MDBKpi cells revealed the presence of viral antigen in over 90 per cent of the cells. Viral antigen was confined to the cytoplasm of the cells, and was often arranged in discrete, perinuclear plaques (Fig. 1a).