Progressive Invasion of Cell Nuclei by Measles Virus in Persistently Infected Human Cells

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SUMMARY

The distribution of measles virus antigens in an auto-degenerating persistently infected human cell line (AV\textsubscript{3}A1/MV) was examined by a direct fluorescent antibody technique. The appearance of virus antigens in cell nuclei increased with time and passage number, and correlated to a decrease in cell replication rates and increases in cellular pathology and mortality. Nuclei which had been invaded by virus antigens were frequently swollen and the cells tended to have round rather than cuboidal morphologies. The correlation between cell degeneration and viral nuclear invasion in this system suggests that quantification of viral nuclear invasion may be useful as a pathological marker of relative cell morbidity in persistent measles virus infections.

Measles virus (MV) replication, like that of other paramyxoviruses, occurs in the cytoplasm of infected cells and results in the accumulation of viral inclusion bodies (Cohen et al., 1955; Milovanovic et al., 1957; Choppin & Compans, 1975). While cytoplasmic viral inclusions are found in both acute and persistent MV infections, nuclear inclusions are found much more frequently in persistent infections (Bouteille et al., 1965; Rustigian, 1966; Martinez et al., 1974). In the slow MV infection of subacute sclerosing panencephalitis (SSPE), nuclear viral inclusions have been so consistently found in brain tissues that they have been proposed as diagnostic markers of the disease (Martinez et al., 1974). Although nuclear virus inclusions have been described in persistent infections in vitro and in SSPE-derived tissues over the last 18 years, the role that nuclear invasion by the virus plays in the development and evolution of such infections is unknown.

In a recent communication, a measles virus-carrier cell line (AV\textsubscript{3}/MV) was described which slowly deteriorated after continuous passage in vitro (Rapp & Robbins, 1981). Deterioration of the cells was characterized by decreased cell replication rates and increased cell spindling and rounding. Fusion was only infrequently observed. The mechanism(s) responsible for the deterioration of the cells was not determined but the intracellular accumulation of large aggregates of virus nucleocapsids was thought to be involved.

In this communication, we describe the distribution of virus-specific antigens in a cloned AV\textsubscript{3}/MV cell line (AV\textsubscript{3}A1/MV) during sequential passage in vitro. The results of our studies show that invasion of AV\textsubscript{3}A1/MV cell nuclei by measles virus progressively increases as the cell cultures degenerate.

The cell line used in these studies was derived from a cloned subline of human amnion cells (AV\textsubscript{3}) which survived acute infection with the Edmonston strain of measles virus. As described previously (Robbins et al., 1980; Rapp & Robbins, 1981), cells surviving the primary infection were grown to confluence and passaged in Eagle's (modified) minimal essential medium (Flow Laboratories) supplemented with 2 mM-L-glutamine, 5 to 10% (v/v) foetal calf serum, 200 units of penicillin and 100 µg of streptomycin per ml (Glaxo Australia). Before use, the pH of the medium was adjusted to 7.2 with sodium bicarbonate. All cultures were maintained at 37 °C. To determine the distribution of virus-specific antigens within the persistently infected cells, cells were grown on coverslips for 48 h, fixed in cold acetone, and assayed by a fluorescent antibody
Fig. 1. Distribution of measles virus-specific antigens in persistently infected human amnion cells (AV3A1/MV) as detected by fluorescent antibody: AV3A1/MV cells 15 weeks after primary infection (a) and 27 weeks after primary infection (c). Parts (b) and (d) show the location of cell nuclei in (a) and (c) respectively after staining with a DNA-specific fluorescent stain (DAPI). Arrows in (c) show nuclei which have been invaded by virus antigens. The three nuclei in the upper left hand corner of (c) were not scored as invaded because of their rounded morphologies (see text). All bar markers represent 5 μm.

technique using fluorescein-conjugated goat antibody to measles virus (Microbiological Associates, Walkersville, Md., U.S.A.). Concurrently, cell nuclei were fluorescently stained with a solution of DAPI (4',6-diamidino-2-phenylindole) in phosphate-buffered saline (Russell et al., 1975). After staining, coverslips were mounted in a Tris-buffered glycerol solution (Sigma) and examined under a Leitz Orthoplan fluorescent microscope at 510 nm (fluorescein) and 470 nm (DAPI). The distribution of antigen was scored as either cytoplasmic or nuclear, based on its apparent location when examined at the two different wavelengths. Because of the difficulties in assessing antigen location in rounded cells, nuclear invasion in such cells was not scored. However, nuclei in rounded cells were included in total nuclei counts (see the legend to Fig. 1).

As shown in Fig. 1(a, b), the intracellular distribution of measles virus antigens in early passages of the AV3A1/MV cell line was predominantly cytoplasmic. The inclusions tended to be found in the perinuclear region, were somewhat pleiomorphic, and usually appeared as discrete rounded bodies of various sizes. While virus antigens were occasionally seen in cell nuclei in the first 12 to 15 passages, the proportion of nuclei showing such invasion sharply increased in the later passages (passages 15 to 25) (Fig. 2). Nuclei that had been invaded in the later passages were frequently swollen and/or misshapen (Fig. 1c, d).
Overt degeneration of the cells occurred over a period of 4 to 6 weeks and in four separate studies over a 19 month period. The degenerative process was characterized by increased rounding and/or spindling of cells, decreased adherence of cells to culture vessels, and failure of the cells to proliferate. As shown in Fig. 3, the AV3A1/MV cells grew more slowly than uninfected AV3A1 cells and tended to stop growing at lower densities. Overtly deteriorating cells proliferated very little when passaged and eventually detached from vessel surfaces (see passage 24 in Fig. 3). Although fusion occurred in the AV3A1/MV cell cultures as in the AV3/MV cells described previously (Rapp & Robbins, 1981), it was not frequently observed (<5% of cells being involved) and did not show any increase in degenerating cultures. After prolonged feeding of deteriorated cultures (3 to 4 weeks), cells would sometimes grow back out and repopulate the culture vessels. Examination of these surviving cells with measles-specific fluorescent antibody and DAPI revealed a low invasion rate in cell nuclei (data not shown).

The possibility that the cell cultures were deteriorating due to mycoplasma contamination was considered; however, such contamination could not be detected in healthy or deteriorating cells when examined with DAPI (Russell et al., 1975) or Hoechst stains (bisbenzimide; Sigma) nor could mycoplasma be detected in thin sections of AV3A1/MV cells examined under the electron microscope.

The results of these studies show that a correlation exists between the extent of viral nuclear invasion and the progression of morbidity in AV3A1/MV cells. Whether the invasion process is directly or indirectly involved in the degeneration of cells was not determined. While the intranuclear accumulation of large viral aggregates may conceivably play a role in cell degeneration (e.g. by interfering with essential nuclear functions), it is also possible that viral invasion occurs after nuclei have become penetrable due to other, perhaps unrelated, pathological processes (i.e. invasion is coincidental).
Short communication

As mentioned earlier, nuclear invasion by measles virus occurs in acutely infected cells, but invasion is much more common in persistent infections (Bouteille et al., 1965; Rustigian, 1966; Oyanagi et al., 1971; Dubois-Dalcq et al., 1974; Martinez et al., 1974). A number of studies have also described morphological and pathological changes in the nuclei or nuclear elements of persistently infected cells. These changes include (i) chromosomal damage (Nichols & Levan, 1965), (ii) changes in chromosome number (Heneen, 1976), (iii) nuclear body formation (Oyanagi et al., 1970; Fournier et al., 1981; Dupuy-Coin et al., 1982) and (iv) changes in nucleolar organization (Heneen, 1978). Whether the pathological nuclear changes that occur in persistently infected cells result from the invasion of cell nuclei by the virus is not clear. Such a connection will ultimately have to be proved by detailed ultrastructural, histochemical and kinetic studies. However, the results of the present study suggest that quantification of virus nuclear invasion may be useful as a pathological marker of relative cell morbidity in persistent MV infections.

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REFERENCES


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