Enhanced Intranuclear Expression of Measles Virus Following Exposure of Persistently Infected Cells to Cyclic AMP

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SUMMARY

The intracellular distribution of measles virus inclusion bodies in persistently infected human cells (AV3A1/MV) changed markedly following continuous exposure to 3',5' cyclic adenosine monophosphate (cAMP). When assayed by immunofluorescence, the number of cells with intranuclear virus inclusions increased from 5 to 10% to 80 to 90% after exposure to 1 mM-cAMP for 4 days. Exposure of cells to cAMP also resulted in a twofold increase in the average number of inclusions in invaded nuclei. Similar but less pronounced changes occurred in cells treated with inducers of adenylate cyclase and an inhibitor of phosphodiesterase. Examination of cAMP-treated cells by electron microscopy indicated that viral inclusion bodies consisted of typical helical nucleocapsids. No evidence of nucleocapsids crossing the nuclear membrane (through nuclear pores) was found.

Measles virus (MV) replication in vivo and in cells experimentally infected in vitro results in the intracellular accumulation of virus inclusion bodies (Enders & Peebles, 1954; Kallman et al., 1959; Sherman & Ruckle, 1958; Tawara et al., 1961). Such inclusion bodies are composed of aggregates of the helical nucleocapsid of the virus, a filamentous structure consisting of the viral genome and at least three viral proteins (NP, P and L) (Bussell et al., 1974; Mountcastle & Choppin, 1977; Robbins et al., 1980a, b; Stallcup et al., 1979; Udem & Cook, 1984). In acutely infected cells, nucleocapsid inclusion bodies first appear in the cytoplasm and subsequently, but to a lesser extent, in the nucleus (Cohen et al., 1955; Llanes-Rodas & Liu, 1965; Nakai & Imagawa, 1969; Raine et al., 1969, 1971, 1973; Feldman et al., 1972). Whether the intranuclear accumulation of nucleocapsids is due to migration of cytoplasmic structures from the cytoplasm or to de novo synthesis (and/or assembly) within the nucleus is not known.

While cytoplasmic nucleocapsid inclusions are found in both acute and persistent MV infections, nuclear inclusions are found much more frequently in persistent infections (Bouteille et al., 1965; Dubois-Dalcq et al., 1974; Oyanagi et al., 1970, 1971; Rustigian, 1962, 1966; Giraudon et al., 1984). For example, such inclusions are found so consistently in brain biopsy and autopsy materials from patients with the MV-associated slow viral disease of subacute sclerosing panencephalitis (SSPE) that they have been proposed as diagnostic markers (Martinez et al., 1974). Concurrent with the appearance of viral inclusion bodies, a number of morphological and pathological changes also occur in the nuclei or nuclear elements of persistently infected cells. The exact relationship between intranuclear expression by the virus and the occurrence of pathological nuclear changes is not clear although a correlation between intranuclear MV expression and cell degeneration has been recently described (Robbins, 1983).

Previous studies (Robbins & Rapp, 1980; Robbins et al., 1984; Dore-Duffy, 1982; Miller & Carrigan, 1982; Yoshikawa & Yamanouchi, 1984) have also shown that MV replication in human cells is significantly affected by cAMP, inducers of adenylate cyclase, and inhibitors of...
phosphodiesterase. These effects include decreased production of infectious particles, inhibition of virus-mediated cell fusion, altered morphology and distribution of cytoplasmic nucleocapsids, impairment of cytoplasmic nucleocapsid and virion assembly, and inhibition of virus-specific protein and RNA synthesis. A number of these processes appear to be similarly affected in the central nervous system (CNS) tissues of SSPE patients as well as in SSPE-derived virus-carrier cell lines (Dubois-Dalcq et al., 1974; Hall & Choppin, 1979, 1981; Lin & Thormar, 1980; Martinez et al., 1974; Oyanagi et al., 1970, 1971). Based on these similarities and the relatively high intracellular levels of cAMP in CNS tissues (Nathanson, 1977), it has been proposed that cAMP (or events mediated by cAMP) may be involved in modulating MV replication in SSPE (Robbins & Rapp, 1980; Miller & Carrigan, 1982).

In the course of examining the inhibitory effects of cAMP on the production of MV-specific proteins in persistently infected cells, we observed significant changes in the intracellular distribution of virus inclusion bodies (Robbins et al., 1984). To define this phenomenon better, we conducted a series of experiments and examined the intracellular distribution of virus inclusion bodies following continuous treatment of persistently infected cells with cAMP, inducers of adenylate cyclase or an inhibitor of phosphodiesterase.

A cloned subline of human amnion cells persistently infected with measles virus (AV3A1/MV) (Robbins, 1983) was used in all experiments. The establishment and characteristics of the parental cell line (AV3/MV) have been described elsewhere (Rapp & Robbins, 1981). All cells were passaged and maintained in Eagle's (modified) MEM (Flow Laboratories) supplemented with 2 mM-l-glutamine, 5% foetal calf serum, 200 units penicillin and 100 μg streptomycin per ml (Glaxo). Prior to use, the pH of all media was adjusted to between 7.2 and 7.4 with sodium bicarbonate. Medium added to experimental cultures was identical to passage medium except the serum concentration was reduced. All cultures were maintained at 37 °C. Cyclic nucleotides, inducers of adenylate cyclase and inhibitors of phosphodiesterase were obtained from Sigma. Solutions used in experiments were prepared using serum-free or low-serum (0.5%, v/v) Eagle's (modified) MEM supplemented as described above. In all experiments, media were replaced daily. The intracellular distribution of viral antigens was determined by direct or indirect immunofluorescence as described previously (Robbins & Rapp, 1980; Robbins, 1983). Cells were grown on glass coverslips and immersed in cold (−20 °C) acetone prior to assay. Nuclei were localized by staining acetone-fixed cells with 4',6-diamidino-2-phenylindole (DAPI) (Russell et al., 1975). Cells on coverslips were fixed with 3% glutaraldehyde in 100 mM-cacodylate buffer, post-fixed in 1% osmium tetroxide, stained en bloc in 2% uranyl acetate and embedded in Spurr's resin. Thin sections were stained with uranyl acetate and lead citrate and examined in a Philips EM 400 electron microscope.

As shown in Fig. 1, AV3A1/MV cells exposed to 1 mM-cAMP and examined by a fluorescent antibody technique demonstrated high levels of virus inclusion bodies within cell nuclei. Although no increase in the number of nuclei containing virus bodies was seen after 1 day, up to 90% of nuclei expressed such materials after exposure for 2 to 4 days (see Fig. 2). Beyond 4 days, cells tended to round up and detach from the coverslips, leaving insufficient numbers of cells to assay (< 500). Mock-treated cells (medium alone) and cells treated with 5'-AMP showed no noticeable increase in the number of nuclei containing such inclusions.

In addition to the increase in the number of nuclei expressing virus inclusion bodies, there was an increase in the number of inclusion bodies within 'invaded' nuclei after cAMP treatment. As shown in Fig. 3, exposure of AV3A1/MV cells to 1 mM-cAMP resulted in an increase of up to twofold in the average number of intranuclear inclusion bodies in invaded nuclei. Although the relative number of inclusion bodies increased after 3 or 4 days, optimal increases were obtained only after longer treatment. The inclusions in such nuclei were frequently found in circular or semicircular arrays and, on occasion, up to 30 discrete inclusion bodies could be found in a single nucleus (data not shown). In such instances, the bodies were generally very small and it was unclear whether they represented newly synthesized materials or fragments of larger bodies.

The number of nuclei containing virus inclusion bodies was also substantially higher in cells treated with two known inducers of adenylate cyclase (serotonin and prostaglandin E₁) (see Table 1). Although the number of nuclei with viral inclusions was not as high as was found in
Fig. 1. Distribution of MV-specific antigens in persistently infected (AV3A1/MV) cells as detected by immunofluorescence after 6 days in the control culture (a) and after exposure to 1 mM-cAMP for 4 days (b), or 6 days (c). Magnification × 1000.

Fig. 2. Rate of MV-specific antigen expression (invasion) in AV3A1/MV cell nuclei following exposure to 1 mM-cAMP. The rate of invasion was determined by scoring a minimum of 500 nuclei under a Leitz Orthoplan fluorescence microscope at a magnification of 400× following treatment of acetone-fixed cells with fluorescein isothiocyanate-conjugated goat anti-MV antibody and DAPI (Robbins, 1983; Robbins et al., 1984; Russell et al., 1975). Nuclei (DAPI-staining regions) in which viral antigens were detected were scored as invaded. ●, cAMP-treated; ○, mock-treated.

Fig. 3. Number of MV-specific inclusion bodies in invaded AV3A1/MV cell nuclei following exposure of cells to 1 mM-cAMP. Each point represents the average number of virus-specific intranuclear inclusion bodies per nucleus based on scoring a minimum of 200 invaded nuclei. The number of intranuclear virus inclusion bodies in each invaded nucleus was determined by photographing invaded nuclei and scoring on enlarged (20 × 25 cm) prints.

Table 1. Invasion of AV3A1/MV cell nuclei by MV-specific antigen following exposure to cAMP, inducers of adenylate cyclase or an inhibitor of phosphodiesterase for 4 days

<table>
<thead>
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<th>Treatment</th>
<th>Nuclei invaded (%)</th>
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<tbody>
<tr>
<td>cAMP (1 mM)</td>
<td>67-2</td>
</tr>
<tr>
<td>Prostaglandin E1 (100 μM)</td>
<td>16-3</td>
</tr>
<tr>
<td>Serotonin (250 μM)</td>
<td>20-6</td>
</tr>
<tr>
<td>Papaverine (2.5 μg/ml)</td>
<td>23-3</td>
</tr>
<tr>
<td>Control (medium only)</td>
<td>6-3</td>
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cAMP-treated cells, a doubling or trebling of the relative number of invaded nuclei could be consistently obtained. With the exception of cAMP itself, papaverine (an inhibitor of phosphodiesterase) seemed to be the most effective inducer of intranuclear virus expression.

When cAMP-treated coverslip cultures of AV3A1/MV cells were thin-sectioned and examined by electron microscopy, intranuclear nucleocapsid aggregates were found in a high percentage of cells. Not uncommonly, more than one aggregate was observed in each nucleus (Fig. 4). The nucleocapsids had 'smooth' morphology and were approximately 18 nm in diameter (Tawara, 1965; Matsumoto, 1966; Oyanagi et al., 1970, 1971). Although nucleocapsids were occasionally seen in peripheral regions of the nucleoplasm, they were usually centrally located and were frequently found in association with nuclear bodies which appeared to be of nucleolar origin.

Nucleocapsids were also consistently found in aggregates in the cytoplasm although seldom found in perinuclear areas. These structures were of the 'fuzzy' or 'granular' type and tended to form tight arrays which included small regions of the cytoplasm (Nc in Fig. 4). No evidence of nucleocapsid penetration of the nuclear membrane via nuclear pores was observed. Similarly, no evidence of nuclear penetration by nucleocapsids during mitosis was observed, as cAMP treatment arrested AV3A1/MV cell division within 24 h (data not shown).

Fig. 4. Transmission electron micrograph of a multinucleated AV3A1/MV cell following exposure to 1 mm-cAMP for 4 days. Encircled areas contain the typical helical nucleocapsids of the virus. N, Nucleus; C, cytoplasm; Nc, cytoplasmic nucleocapsid aggregate. Bar marker represents 1 μm. Inset: enlargement of the boxed area in the figure showing the nucleocapsids of the virus in more detail (bar marker represents 500 nm).
The results of these experiments have provided evidence that the intranuclear expression of MV is enhanced following exposure of persistently infected AV3A1/MV cells to cAMP. This evidence includes a progressive increase in the relative number of cell nuclei containing virus-specific inclusion bodies, an increase in the average number of such inclusion bodies in the nuclei, and an increase in the number of intranuclear virus nucleocapsid aggregates following exposure of cells to medium containing the chemical. Similar but less pronounced increases were also observed in cells treated with two known inducers of adenylate cyclase (prostaglandin E$_1$ and serotonin) and an inhibitor of phosphodiesterase (papaverine). Although the exact mechanism by which intranuclear virus expression is enhanced was not determined, our findings suggested that elevation of the intracellular levels of cAMP is involved. Based on the distribution of intranuclear nucleocapsid aggregates and the absence of nucleocapsids breaching the nuclear membrane (e.g. through nuclear pores), transmigration of cytoplasmic nucleocapsids does not appear to be responsible.

cAMP markedly affects the replication of measles virus in infected human and primate cells (Dore-Duffy, 1982; Miller & Carrigan, 1982; Robbins & Rapp, 1980; Yoshikawa & Yamanouchi, 1984). A number of other studies have shown that adenylate cyclase-stimulating anaesthetics also inhibit measles virus replication (Knight et al., 1980, 1981; Triner et al., 1977). Taken together, these data suggest that the intracellular levels of cAMP may play a modulatory role in determining the outcome of measles virus infections. However it should be remembered that in AV3/MV cells the intracellular cAMP concentration is normally 1 μM (unpublished data). Nevertheless given the similarities between the phenomena occurring in cAMP-treated MV-infected cell systems and the biomolecular and virological processes found in SSPE, it is possible that cAMP, or events that it mediates, are involved in the pathogenesis of the disease.

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**REFERENCES**


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