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Measles virus infection of cells in perivascular infiltrates in the brain in subacute sclerosing panencephalitis: confirmation by non-radioactive in situ hybridization, immunocytochemistry and electron microscopy

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Summary. As part of continuing multidisciplinary studies on the neuropathogenesis of subacute sclerosing panencephalitis (SSPE), in situ hybridisation, immunocytochemistry and electron microscopy were used to detect measles virus nucleic acid, protein and nucleocapsids in brain perivascular infiltrates of three cases. Perivascular cuffing cells which contained measles virus nucleic acid and antigens were found in all cases. Infected cuffs occurred predominantly in areas of general parenchymal cell infection and in many of these a high proportion of the infiltrating cells were infected. Other cuffs in these areas were either uninfected or contained only a few infected cells. Occasional infected cells were also seen in cuffs in non-infected areas. In contrast, no specific immunocytochemical reactions or in situ hybridisation for measles virus was observed in brain tissue from a patient with herpes encephalitis. By electron microscopy viral nucleocapsid, consistent with measles virus, was found within the cytoplasm of plasma cells in the inflammatory cuffs in SSPE brain tissue. Possible explanations for our results are that infiltrates become infected on arrival in the CNS or alternatively, that the infected infiltrates reflect a generalised infection of the reticuloendothelial system. The frequent presence of uninfected cuffs favours the former explanation.

Key words: Measles – Subacute sclerosing panencephalitis – Perivascular infiltrates – Plasma cells

Subacute sclerosing panencephalitis (SSPE), a slowly progressive, fatal disease of the human CNS, is caused by a persistent infection with measles virus (MV) [4]. Pathologically, SSPE is characterised by the presence of measles inclusion bodies in neurons and glia, by demyelination and by a mild to moderate inflammatory response with perivascular cuffs and tissue infiltration by lymphocytes and macrophages [18].

Immunocytochemical (ICC) and in situ hybridisation (ISH) studies of the CNS in SSPE have demonstrated MV antigens and genome, mainly within neurons and oligodendrocytes [6, 14]. However there is also a single case report of a study in which MV RNA sequences were detected by radioactive ISH in "numerous infiltrating cells, predominantly lymphocytes" in perivascular cuffs in the CNS [7]. However, immunofluorescence screening for MV antigen was negative in these cuffs.

In a recent study in this laboratory, in which SSPE CNS tissue sections served as positive controls, hybridisation was observed in some perivascular infiltrates of inflammatory cells, when using an ^{35}S-labelled probe for the MV N gene [5]. These early incidental findings left several questions unanswered about the nature and extent of the infection and about its possible pathogenetic significance. A more thorough study of this issue is now particularly appropriate, following the recent finding in our laboratory of MV infection in cerebral endothelial cells in SSPE [11].

The present report describes the results of a study of the occurrence of MV in perivascular infiltrates in post-mortem CNS tissues from three cases of SSPE. For these investigations optimised, highly sensitive ICC and ISH techniques, previously developed in this laboratory, were used [12, 13]. Transmission electron microscopy (TEM) was used in one case to confirm the presence of nucleocapsids and to identify the infected cells.

Materials and methods

Tissue sources

CNS tissue from three cases of SSPE were studied. Control necropsy tissue comprised normal brain tissue and brain tissue from a case of herpes simplex virus I encephalitis with widespread perivascular inflammation. A large number of brain blocks from the three SSPE cases were processed to paraffin. Sections (4 μm) from all blocks were cut onto 3-amino-propyltriethoxysilane (APES)-treated slides.

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Immunocytochemistry

Following dewaxing, rehydration and blocking of endogenous peroxidase, sections were treated with proteolytic enzyme (protease VIII Sigma; 0.5 mg/ml in PBS 5 min room temperature). Following incubation in 5% normal goat serum for 20 min, sections were incubated in 100 µl of a hyperimmune SSPE serum (1/1000 in 10 mM PBS) overnight at 4 °C. Antibody-antigen reactions were detected using a biotin-streptavidin method [13].

In situ hybridisation and immunodetection of biotinylated hybrids

Biotinylated single-stranded RNA probes to the N gene of MV were prepared by subcloning the N gene sequence into a gemini in vitro transcription vector. A sequence derived from this vector was used as a control probe. For ISH, sections were dewaxed, rehydrated, blocked for endogenous peroxidase and treated with proteolytic enzyme (protease VIII at 0.5 mg/ml in PBS 10 min room temperature). Hybridisation and immunodetection of the biotinylated hybrids was carried out as described elsewhere [12].

Electron microscopy

Tissue was taken from formalin storage, refixed in 2.5% buffered glutaraldehyde, post-fixed in osmium tetroxide, dehydrated, embedded in epoxy or acrylic resin, sectioned and stained for electron microscopy, as described previously [11].

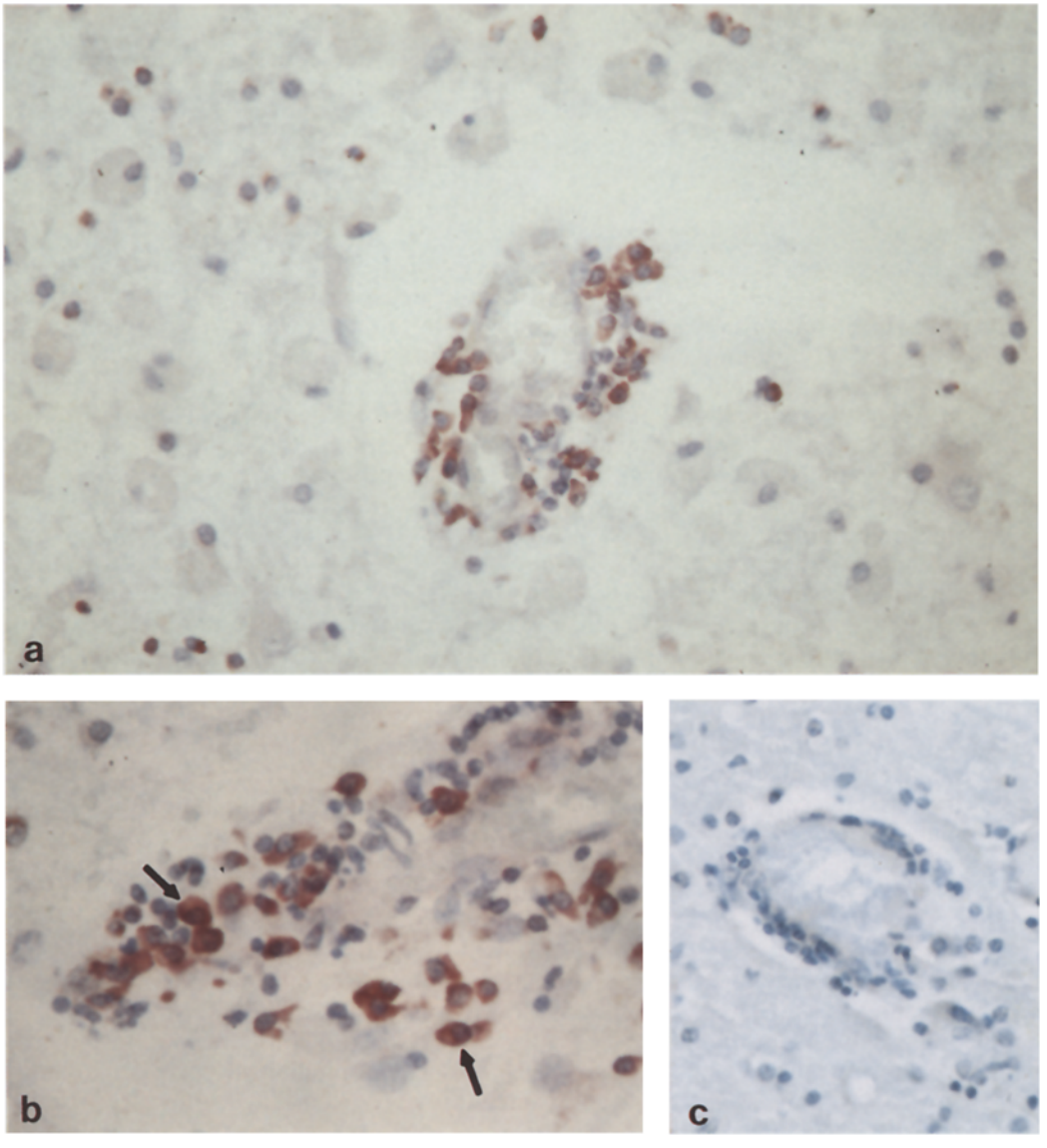

Fig. 1. a Measles virus (MV) hybridisation with biotinylated probe to MV N genomic RNA sequence in perivascular cuffing cells in subacute sclerosing panencephalitis (SSPE) brain. Note infection within surrounding parenchymal cells. **b** High magnification of another cuff shows the varied morphology of the MV-positive cells, which include some resembling plasma cells (*arrows*). Note also that many cells are negative for this probe. **c** MV-specific biotinylated probe does not hybridise with cuffing cells in sections from a case of herpes simplex virus I encephalitis. **a,c** × 250; **b** × 800

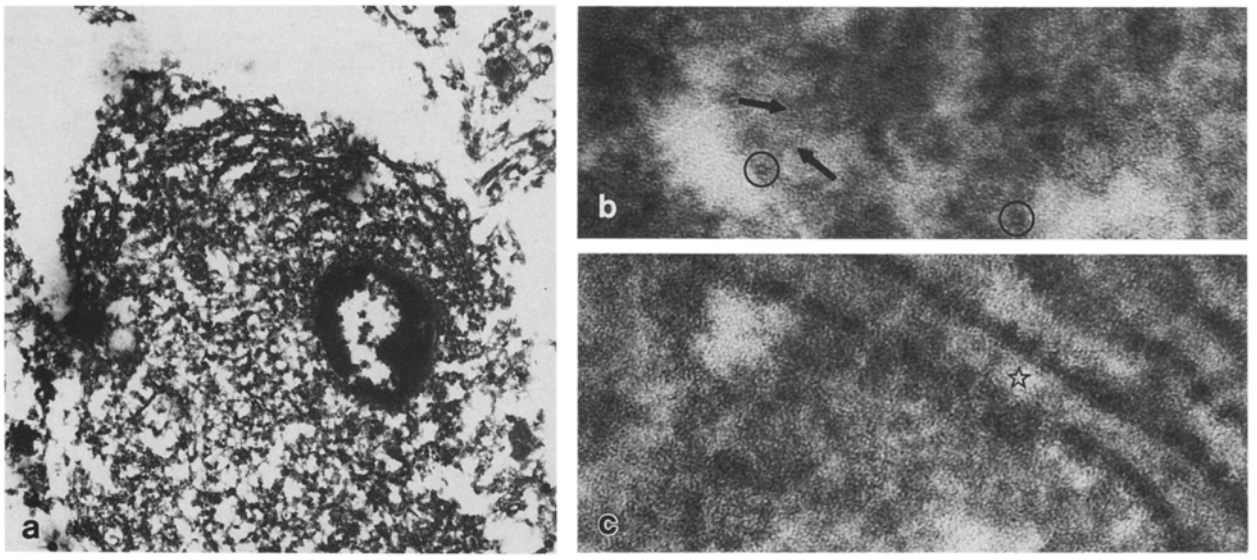

Fig. 2a–c. Ultrastructure of tissues rescued from long-term formalin storage. **a** A cuffing plasma cell shows no well-defined inclusion body, but the rough endoplasmic reticulum is confined mainly to one pole. **b** Cuffing cell: cytoplasmic nucleocapsids consistent with measles virus are seen either in transverse section (*circles*) or longitudinally (barely visible sinuous structure running from *circle* and between *arrows*). **c** The same cell contains extensive rough endoplasmic reticulum (*asterisk*). **a** × 8,100; **b,c** × 132,000

Results

Neuropathological examination of the three necropsied cases revealed the features typical of SSPE, including the presence of perivascular mononuclear cell infiltrates. Using both ICC and ISH, MV-positive brain cells were detected in numerous brain blocks from all three SSPE cases. While the extent of infection varied between cases the following generalised findings were noted. In the parenchyma the infection was predominantly in neurons and oligodendrocytes, with only a few positive macrophages and astrocytes. A consistent finding in all three cases was the presence of perivascular cuffing cells which contained MV antigens and nucleic acid (Fig. 1a,b). Many though not all, of these infected cells, which were found in a large number of cuffs, resembled plasma cells (Fig. 1b). Infected cuffs were found predominantly in areas of marked parenchymal infection, though cuffs without detectable virus-infected cells were also noted in these areas. Occasional infected cells were also seen in cuffs in non-infected areas. The percentage of positive cells in the cuffs was variable ranging from just 1%–2% to > 90% of the cells comprising the infiltrate.

Although ultrastructural preservation was very poor as a result of prolonged formalin storage and associated factors, viral nucleocapsid, indistinguishable from that seen in neurons and oligodendrocytes in the same sections, were found within the cytoplasm of some plasma cells in the inflammatory cuffs in the case examined (Fig. 2). However, distinct inclusion bodies, which were so prominent in neurons and oligodendrocytes both in semithin toluidine blue-stained sections and in the electron microscope, were never seen in cuffing cells.

The specificity of the ICC and ISH procedures was confirmed by the negative results obtained: (i) when brain sections from the three SSPE cases were reacted with a control normal human serum or hybridised with the gemini vector control probe; or (ii) when sections from normal brain and herpes encephalitis brain were reacted with the MV-specific antiserum or hybridised with the MV-specific probe (Fig. 1c).

Discussion

In the present microscopic study of sections of brain and spinal cord from three autopsied cases of SSPE both antigen and nucleic acid of MV have been detected in perivascular cuffing cells by the use of optimised ICC and ISH techniques [12, 13]. The specificity of reactions was confirmed using vector sequence probes and by the absence of signals on tissues from both normal and pathological control cases. In one case the ICC and ISH evidence was confirmed by the electron microscopic demonstration of viral nucleocapsid in plasma cells within cuffs. This study in which cytologically superior, non-radioactive protocols were used confirms and extends earlier reports in which radioactive methods were employed [5, 7]. Detection of MV antigen in cuffing cells in SSPE has previously been reported only in rare cells within leptomeningeal perivascular infiltrates [3] and was not reported at all in other ICC studies of the CNS [2, 6, 8].

It is noteworthy that distinct inclusion bodies, such as are found in neurons and oligodendroglia, cannot be clearly seen in the infected inflammatory cells in SSPE. In this respect the inflammatory cell infection more closely resembles that seen in cerebral endothelial cells

in SSPE and its characteristics remain to be defined [11]. For example, it is not known whether all viral proteins are produced normally or whether maturation and budding can occur in these cells. By contrast, it is known that in the neurons and oligodendrocytes there is a variable degree of down-regulation of the production of some proteins, most notably the matrix or M protein, though the production of nucleocapsid continues [9]. Consequently, with the observed failure of budding, viral inclusions accumulate in these cells [15]. Unfortunately, in the present study it was not possible to use antibodies to either the different viral proteins or cell-specific markers to examine the infected infiltrates, as these were unreactive in our tissues which had undergone prolonged storage in formalin prior to paraffin-embedding.

Arguably the most important issue arising from these results is the question of whether the inflammatory cells carried the infection from the bloodstream into the cuffs or alternatively became infected secondarily, following their arrival. In models of encephalitis caused by other paramyxoviruses [Newcastle disease virus and canine distemper virus (CDV)], following infection by natural routes and spread by haematogenous route, infected inflammatory cuffs are observed early in the course of the disease [1, 17, 20]. In their study of experimental CDV infection by intraperitoneal inoculation in gnotobiotic beagle dogs, Axthelm and Krakowka [1] first detected viral antigen within CNS capillary and venular endothelia, pericytes and perivascular astrocytic foot processes. Infiltrations in that model were shown to follow endothelial cell infection by 1–2 days and were composed of both viral antigen-positive and antigen-negative cells.

The situation in established SSPE, however, is quite different from that found in the early stages of these experimental models and exact parallels cannot be drawn. In SSPE, observations of infected infiltrates have so far been confined to a time when the virus is already present in massive quantities within the CNS parenchyma. Furthermore, the question of whether circulating leucocytes are infected in SSPE is unresolved; for example, claims that MV RNA sequences were present in 70 %–90 % of peripheral blood mononuclear cells in three cases of SSPE [7] were not confirmed in a recent study [16]. However, MV RNA or antigens or both were recently detected in lymphoid organs including thymus, spleen and lymph nodes, in SSPE at post mortem [2].

The constant observation in the present study of an apparent association between infected cuffs and surrounding infected parenchyma suggests the possibility that in established SSPE infiltrating cells may not become infected until after their arrival in infected areas within the CNS. Irrespective of the origin of the infection, once the virus has reached the perivascular compartment, it would have access to many cells, including T and B cells and monocytes, which are susceptible to MV infection [10, 19].

It is conceivable, therefore, though not proven in this study, that infection of inflammatory cuffing cells in SSPE can occur from the adjacent infected parenchyma and not just as a consequence of the arrival of infected cells from the bloodstream. The significance of infected cuffs in the pathogenesis of SSPE is, therefore, likely to vary depending on the time at which it occurs. Regrettably we have no information on their occurrence prior to or during disease onset.

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