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Multiplication of rubella and measles viruses in primary rat neural cell cultures: relevance to a postulated triggering mechanism for multiple sclerosis

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Rubella virus multiplied to low titre and produced a partial cytopathic effect in rat glial cell cultures. Anti-galactocerebroside staining showed that this cytopathic effect involved the disintegration of oligodendrocytes. A similar effect was produced following infection of myelinating neural cell cultures with rubella virus, but virus multiplication could not be detected in pure neuron cultures. Measles virus was found to multiply and produce a cytopathic effect in primary cultures of both neurons and glial cells. These results are discussed in relation to the ability of measles and rubella viruses to trigger human multiple sclerosis.

Keywords: rubella virus, measles virus, neural cell culture, multiple sclerosis

INTRODUCTION

Multiple sclerosis (MS) is a chronic demyelinating disease of the human central nervous system (CNS). The demyelination occurs in discrete plaques of the CNS, and is immune-mediated. Although it has long been postulated that a virus may be involved in the aetiology of MS, definitive proof of this has not been obtained. It is clear, however, from epidemiological studies, that the event which triggers MS occurs many years before symptoms of the disease become manifest. In the absence of any other plausible hypothesis for the aetiology of MS, the viral theory remains a possibility (Waksman & Reingold, 1986). One difficulty in establishing a viral connection with MS is that the virus may only trigger the disease, and disappear before biopsy and autopsy specimens are available. It has, however, recently been suggested that one or more of the components of the measles, mumps, rubella vaccine may be involved in triggering MS (Alvord, 1989).

We have developed Semliki Forest virus (SFV) infection of mice as a model of virus-induced demyelination and have used both *in situ* hybridization and immunocytochemistry to study SFV infection (Smyth *et al.*, 1990). We have also developed the use of neural cell cultures to

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study the cell tropism of defined strains of SFV and the mechanism of demyelination (Gates *et al.*, 1985; Atkins, Sheahan & Mooney, 1990b). Other animal models of virus-induced demyelination, such as Theiler's virus infection of mice (Rodriguez, Oleszak & Leibowitz, 1987) and murine coronavirus infection of mice and rats (Buchmeier, Dalziel & Koolen, 1988; Massa, Wege & Ter Meulen, 1986b), have shown that demyelination is associated with virus persistence. Because of this, work aimed at associating human viruses with MS has centred on this possibility. Our recent work using the Semliki Forest virus (SFV) mouse model has indicated that demyelination may persist in the absence of virus persistence (Smyth, Sheahan & Atkins, 1990). Persistent demyelination may be induced in this way in the SJL mouse strain which carries a T-suppressor defect, and is susceptible to experimental allergic encephalomyelitis (Lando, Teitelbaum & Arnon, 1980; Hood, Steinmetz & Malissen, 1982).

Measles and rubella viruses have long been implicated in MS, although the results are equivocal (Cosby *et al.*, 1989; Nath & Walinsky, 1990). Both viruses cause common acute infections and a rare encephalitis which involves demyelination (Johnson, 1982). Rubella virus, like SFV, is a togavirus and its molecular biology is similar to SFV. Measles virus is an RNA-containing paramyxovirus. Both viruses have been previously shown to infect rat CNS cells (Schneiderschaulies *et al.*, 1990; van Alstyne *et al.*, 1984).

In this study, we have utilized the rat neural cell culture system, described previously for SFV (Atkins *et al.*, 1990b), to study the cell tropism of rubella and measles viruses, and to relate this to the possible potential of these viruses to trigger MS.

MATERIALS AND METHODS

Virus

The Therien strain of rubella virus and the Edmonston strain of measles virus were grown and plaque assayed using Vero cells, as previously described (Hearne, O'Sullivan & Atkins, 1986). Stocks were frozen in aliquots at −70°C, and infection experiments were carried out at a multiplicity of infection of 10^{-2}. For infection of glial cells and neurons, virus adsorption was allowed for 1 h at 37°C in a volume of 0.2 ml, before the addition of 1 ml of growth medium. Anti-measles antiserum was a gift from Dr L. Cosby, Medical Biology Centre, Queen's University, Belfast, and anti-rubella antiserum from Professor I. Hillary, Department of Medical Microbiology, University College, Dublin; both were human sera.

Preparation of primary CNS cells

Neuron and glial cell cultures were prepared from neonatal rat brain as previously described (Gates *et al.*, 1985; Atkins *et al.*, 1990b). For virus growth curve experiments, 35 mm tissue culture dishes were used; at the time intervals post-infection specified, the medium was removed and stored at −70°C for subsequent plaque assay, the cells were washed 3 times with phosphate buffered saline, and the medium replaced. For immuno-gold silver staining (IGSS), Lab-Tek 4802 2-chamber tissue culture slides were used (Nunc Inc., Naperville, Illinois, USA). The method used was the indirect staining method described previously (Atkins *et al.*, 1990b), and gives a yellow, brown or black colouration. Mouse monoclonal antibody to galactocerebroside (anti-GC; Ranscht *et al.*, 1982) was a gift of Dr B. Ranscht, La Jolla Cancer Research Foundation, La Jolla, California, USA. It was used at a dilution of 1/20. Myelinating cultures were set up as for neurons, but the concentration of cytosine arabinoside was reduced from 10 μg/ml to 5 μg/ml, allowing the survival of some glial cells.

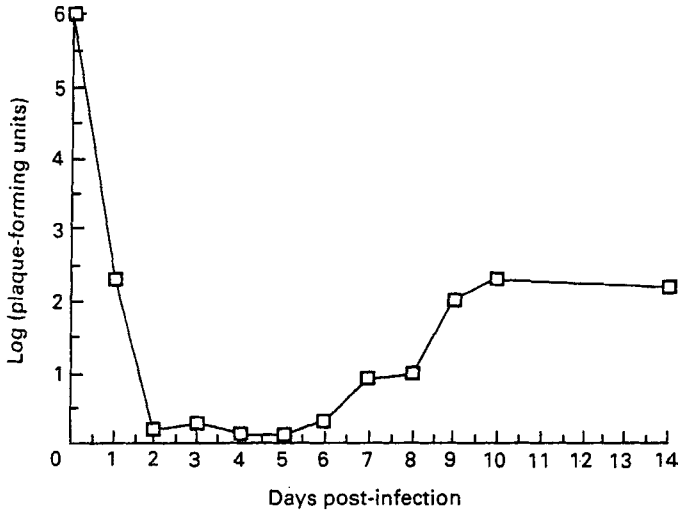


Figure 1. Multiplication of rubella virus in cultured rat glial cells. Each time point represents accumulated virus from the previous time point. Only partial cytopathic effect occurred in these cultures.

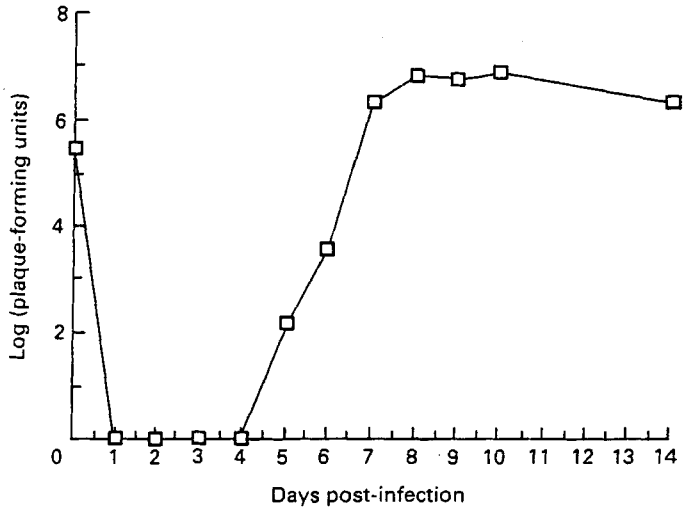


Figure 2. Multiplication of measles virus in cultured glial cells. Each time point represents accumulated virus from the previous time point. Complete cytopathic effect had occurred by 14 days post-infection.

RESULTS

Virus multiplication in glial cells

Both rubella and measles viruses multiplied in cultured glial cells. For rubella virus, multiplication was first detected as an increase in titre at 6 days post-infection (Figure 1), whereas for measles virus this occurred at 5 days post-infection (Figure 2). For rubella virus,

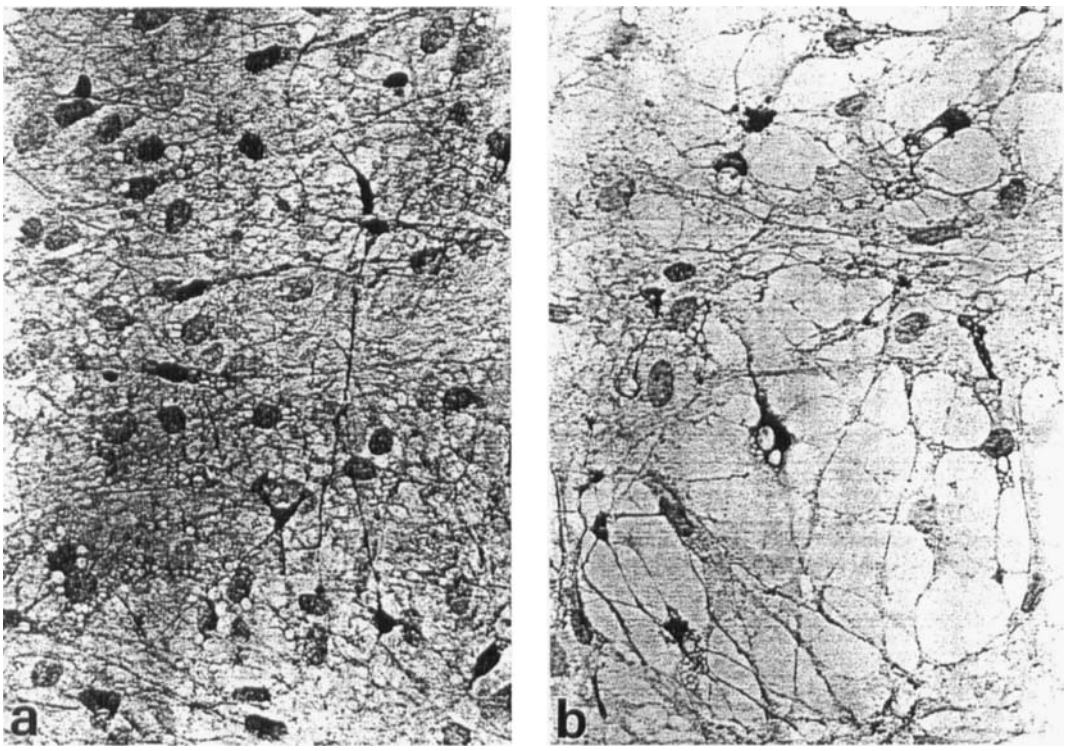


Figure 3. Cytopathic effect induced by measles virus infection of cultured rat glial cells. **a**, Uninfected glial cells 22 days after plating. **b**, Glial cells infected with measles virus at 10 days post-infection and the same time of culture. The spaces between cells represent partial disintegration of the culture. × 400.

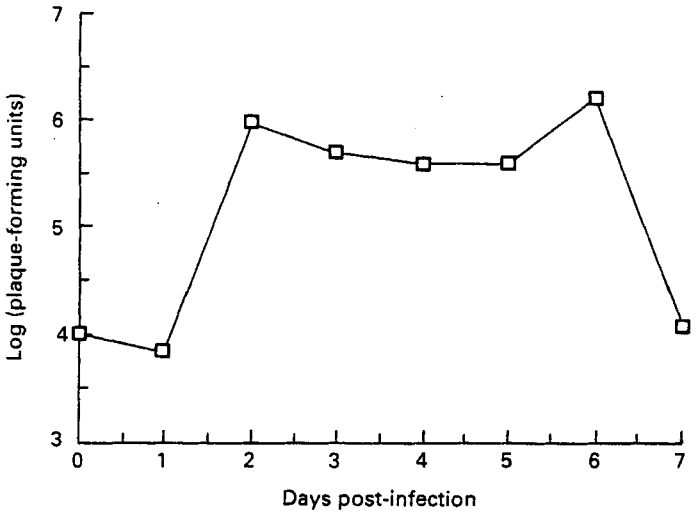


Figure 4. Multiplication of measles virus in cultured rat neurons. Each time point represents accumulated virus from the previous time point. Complete cytopathic effect had occurred by 7 days post-infection.

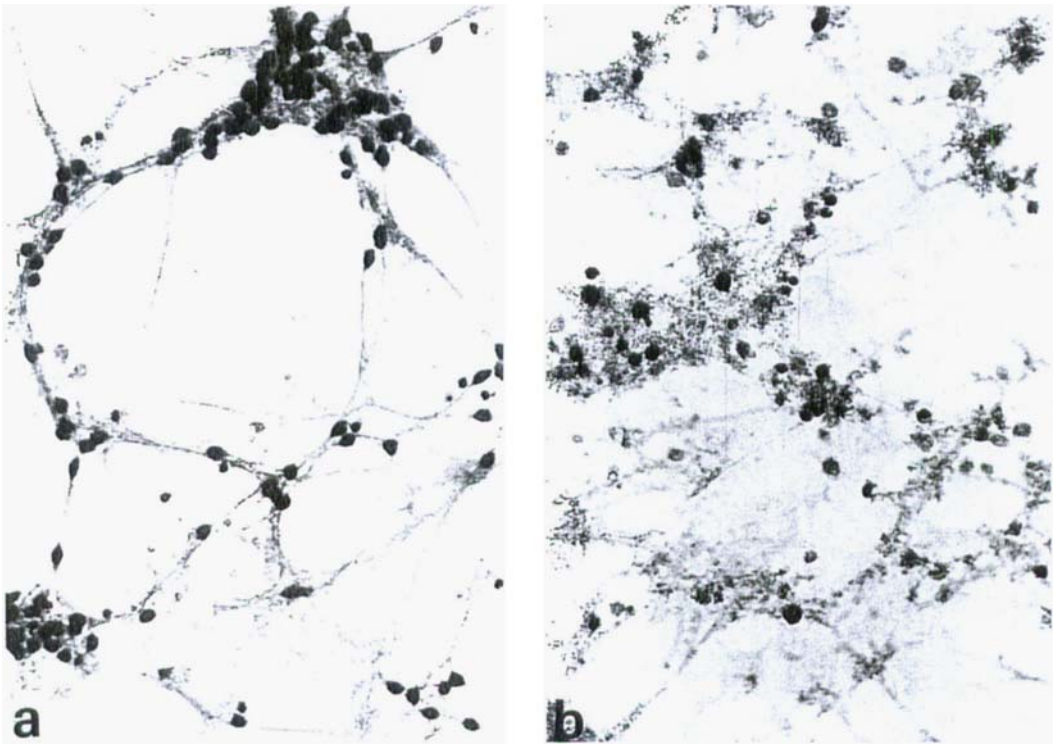


Figure 5. Cytopathic effect induced by measles virus infection of cultured rat neurons. **a**, Uninfected neurons 13 days after plating. **b**, Neurons infected with measles virus at 7 days post-infection and the same time of culture. Note the withdrawal of cell processes and the formation of cellular debris. × 400.

multiplication in glial cells was accompanied by slow lysis of a proportion of cells in the culture (see below). For measles virus, a typical cytopathic effect became apparent (Figure 3), which eventually affected the whole culture.

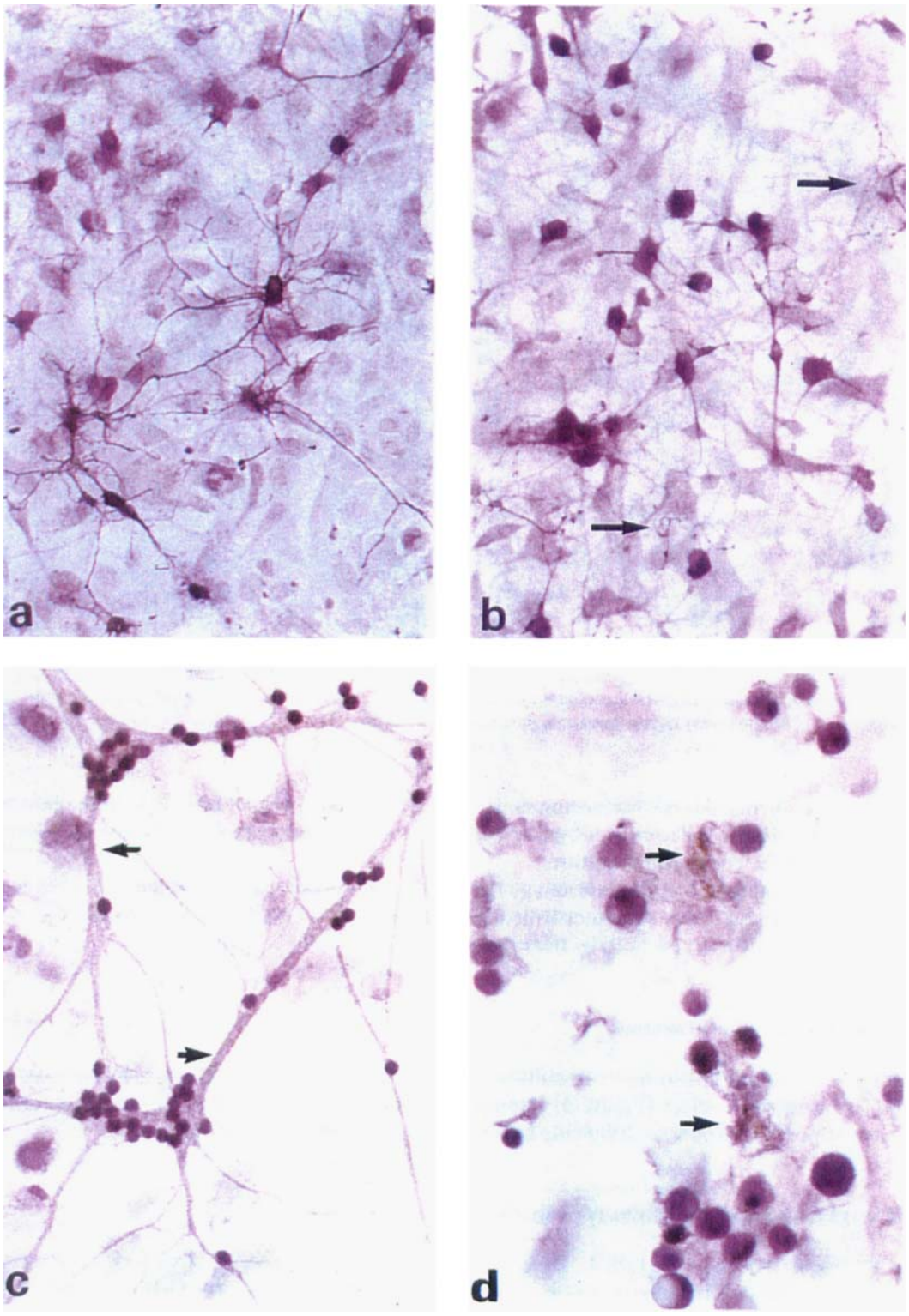
Some staining of measles-infected glial cells with anti-measles antiserum was possible, but such staining could not be demonstrated for rubella virus. This probably reflected the low infectious virus titres produced by the rubella-infected glial cells (Figure 1).

Virus multiplication in neurons

Measles virus multiplied faster in cultured neurons than in glial cells (Figure 4), and gave a typical cytopathic effect (Figure 5). However, no multiplication could be detected, and no cytopathic effect produced, following infection of neurons with rubella virus.

Anti-galactocerebroside antibody (Anti-GC) staining

This was carried out for rubella virus infection of glial cells, and for myelinating cultures. Uninfected glial cells showed typical staining of oligodendrocytes (Figure 6**a**). Infection with


a
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rubella virus caused the slow accumulation of oligodendrocyte damage, which resulted in the generation of anti-GC staining debris and, after washing, the eventual disappearance of anti-GC staining (Figure 6**b**). Infection of myelinating cultures (Figure 6**c**) with rubella virus resulted in the slow disruption of the culture over 6–8 days, and the generation of anti-GC staining debris (Figure 6**d**). During this process, some cells remained intact and small amounts of infectious virus (10^1–10^2 plaque-forming units/ml) were released.

DISCUSSION

This study has shown that rubella virus multiplies to low titre in glial cell cultures derived from rats, and induces damage to oligodendrocytes and myelin. This was shown using antibody to galactocerebroside, an oligodendrocyte marker and myelin component. In this respect, rubella virus is similar to SFV (Atkins *et al.*, 1990b), also a togavirus, and which also induces early damage to oligodendrocytes following infection of mixed glial cell cultures. Unlike SFV, however, rubella virus does not multiply in rat neurons.

In this study, myelinating CNS cultures were obtained by reducing the concentration of cytosine arabinoside, a cytotoxic drug used in the preparation of pure neuron cultures to inhibit the growth of glial cells. A similar result was obtained by Walker, Chapman and Rumsby (1985), who obtained myelinating cultures by the physical disruption of cultured rat brain cells by shaking. We have found that omission of cytosine arabinoside inhibits myelination, probably by overgrowth of the culture by protoplasmic astrocytes; reduction in concentration allows the survival of some oligodendrocytes which are able to form myelinated fascicles. Infection of such cultures with rubella virus disrupts the fascicles and leads to the generation of myelin debris.

Measles virus multiplied in both glial cell and neuron cultures, and induced a typical cytopathic effect in both. This study therefore provides no evidence for selective damage to oligodendrocytes following infection with measles virus. In the human CNS, however, the situation may be different. Interaction of the virus with the immune system, including antibody-induced modulation (Liebert *et al.*, 1990), may restrict the multiplication of the virus.

Based on our previous work with SFV, we postulated that autoimmune demyelination may be induced by presentation of myelin antigens to T-helper lymphocytes through the generation of myelin debris. Such stimulation of T-helper cells may occur by molecular mimicry, that is immune cross-reactions between virus and host components (Oldstone, 1987). To explain the apparently conflicting evidence and hypotheses regarding MS, we propose the mechanism shown in Figure 7, which is based on work carried out by many authors and has been proposed before in different forms. Central to this mechanism is the presentation of myelin antigens to

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Figure 6. Anti-galactocerebroside antibody (anti-GC) staining of rat neural cell cultures using the immuno-gold silver staining (IGSS) technique. Positive staining gives a yellow, brown or black colour. **a**, Mock infected glial cells showing positive staining of oligodendrocytes at 22 days after plating. **b**, Glial cells 22 days after plating and 10 days post-infection with rubella virus, showing relative loss of staining and generation of anti-GC staining debris (arrows). **c**, Myelinating cultures, 23 days after seeding. Note the presence of anti-GC staining fascicles (arrows). **d**, Myelinating cultures, 23 days after seeding, but infected with rubella virus on day 16. Note the severe disruption of the fascicles and the generation of anti-GC staining debris (arrows). Counterstained with haematoxylin. × 400.

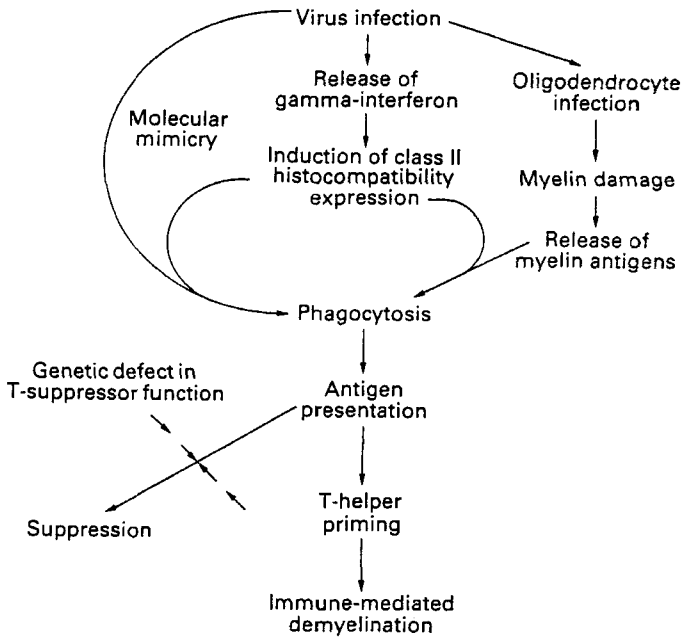

Figure 7. Postulated mechanisms of pathogenesis of multiple sclerosis. The dotted arrows represent a suggested genetic and partial immunological defect in MS patients.

T-helper cells in individuals who carry a genetic defect in T-suppressor cell function. Presentation of such antigens could be carried out by macrophages, and possibly also by other cells such as astrocytes. This antigen presentation occurs in association with type II histocompatibility molecules, which are recognized by the CD4 receptor on T-helper cells. The expression of type II histocompatibility molecules is augmented by the secretion of gamma-interferon, which is produced by T-lymphocytes during virus infection in response to antigenic stimulation. The expression of type II histocompatibility molecules may also be induced directly by virus infection (Massa, Dörries & Ter Meulen, 1986a). Although there has been controversy about whether MS patients show immunity to myelin antigens, it has recently been shown that T-helper cells from MS patients proliferate in response to myelin peptides, if these are presented by macrophages in conjunction with HLA-DR (human type II histocompatibility) molecules (Baxevanis *et al.*, 1989).

The scheme shown in Figure 7 involves triggering of autoimmune demyelination, and does not assume virus persistence or the involvement of any specific virus or agent. According to this hypothesis, sensitization to myelin antigens may occur by molecular mimicry, or by release of myelin antigens following damage to oligodendrocytes. We have obtained evidence to suggest that rubella virus could trigger autoimmune demyelination by generation of myelin debris (this study), or by molecular mimicry (Atkins *et al.*, 1990a). We have shown that there is a sequence similarity between the amino-terminal of myelin proteolipid protein, and rubella envelope E1 protein (Atkins *et al.*, 1990a). Although the present study does not provide evidence for selective damage to oligodendrocytes by measles virus, there is sequence similarity between measles virus and the same amino-terminal sequence of myelin proteolipid proteins (Atkins *et al.*, 1990a).

Measles virus proteins also show sequence similarity to peptides from myelin basic protein (Jahnke, Fischer & Alvord, 1985).

In the work described here, we have used rats to study the tropism of rubella and measles viruses for CNS cell cultures. It is now possible to culture human CNS cells from autopsy tissue, and such cultures contain oligodendrocytes (Newcombe, Meeson & Cuzner, 1988). Therefore it should be possible to test the cell tropism of human viruses using human CNS cells.

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