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Murine coronavirus infection: a paradigm for virus-induced demyelinating disease

Thomas E. Lane and Michael J. Buchmeier

A variety of neurological diseases in humans, including multiple sclerosis (MS), have been postulated to have a viral etiology. The use of animal models provides insights into potential mechanism(s) involved in the disease process. The murine coronavirus-induced demyelinating disease in rodents is one such model for demyelinating disease in humans.

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Current hypotheses to explain the etiology and pathogenesis of demyelinating diseases in humans include the idea that an infectious agent encountered early in life may prime or trigger a disease process that manifests later in life as white-matter demyelination[1]. Although epidemiological evidence points to an infectious etiology, a single agent has never been linked convincingly with human diseases such as multiple sclerosis (MS), a chronic central nervous system (CNS) disease that is characterized by multifocal inflammatory foci and myelin destruction[2,3]. Animal models of virus-induced demyelination have provided useful paradigms to study the demyelinating process.

Coronaviruses constitute a large group of positive-stranded RNA viruses that are associated with a wide variety of respiratory, gastrointestinal and neurological diseases in animals and humans[4,5]. Although the majority of coronavirus infections of humans are associated with upper respiratory tract infections, several laboratories have attempted to correlate inflammatory neurological disease with coronaviral infection, and recent reports have demonstrated the presence of coronavirus RNA and antigens in demyelinating plaque lesions in the brains of MS patients[6,7]. Furthermore, increased levels of antibodies against coronavirus have been detected in MS patients, as compared with control patients[8], and a murine coronavirus [mouse hepatitis virus (MHV)], which had previously been thought only to infect mice, has recently been shown to replicate and cause demyelination in the CNS of nonhuman primates[9]. At this time, however, there is no conclusive evidence that any human neurological disease, let alone MS, occurs as a result of human coronavirus infection. Certainly, other viruses, including herpes simplex virus type-1 (HSV-1)[10], measles[11] and human T cell leukemia virus type 1 (HTLV-1)[12], have been suggested to be associated etiologically with MS (Ref. 13). However, the accumulated data suggest that the human coronaviruses are capable of infecting both the human and nonhuman primate CNS. Given the existing experimental evidence for murine coronavirus involvement in virus-induced CNS disease in rodents, the possibility that the human coronaviruses are capable of causing a similar encephalomyelitis in humans remains open. Clearly, the results indicate that the host range and potential to cause disease by coronaviruses are more complex than appreciated previously and reinforce the need for a better understanding of the biology of human and mouse coronaviruses[9].

The murine coronaviruses are capable of causing a variety of diseases in mice, such as hepatitis and gastroenteritis[4]. Infection of rodents with neuroadapted strains of MHV results in acute encephalitis and white-matter demyelination[4,5]. There are several similarities between MHV-induced demyelination and MS, which make this a good laboratory model for studying the underlying mechanisms of the MS disease process (Table 1)[14–16]: (1) genetic susceptibility appears to play a prominent role in the development of MS in humans while the genetic background of rodents, which determines susceptibility and immune response, is of crucial importance in MHV-induced demyelination[3,14–17]; (2) MS patients often experience cyclic periods of exacerbation followed by remission[3,18], and MHV-infected

Table 1. Comparison of pathological events in multiple sclerosis (MS) and coronavirus demyelination

Multiple sclerosis	Coronavirus
Infectious agent suggested by epidemiology	Viral trigger
Genetic susceptibility	Genetic susceptibility
Multifocal white-matter lesions	Multifocal white-matter lesions
Exacerbation of disease symptoms	Subacute, acute and chronic stages
Myelin stripping	Myelin stripping
NOS-2 expression	NOS-2 expression
IFN-γ	IFN-γ
IL-1	IL-1
TNF-α	TNF-α

Abbreviations: IL-1, interleukin 1; IFN-γ, interferon γ; NOS-2, nitric oxide synthase; TNF-α, tumor necrosis factor α.

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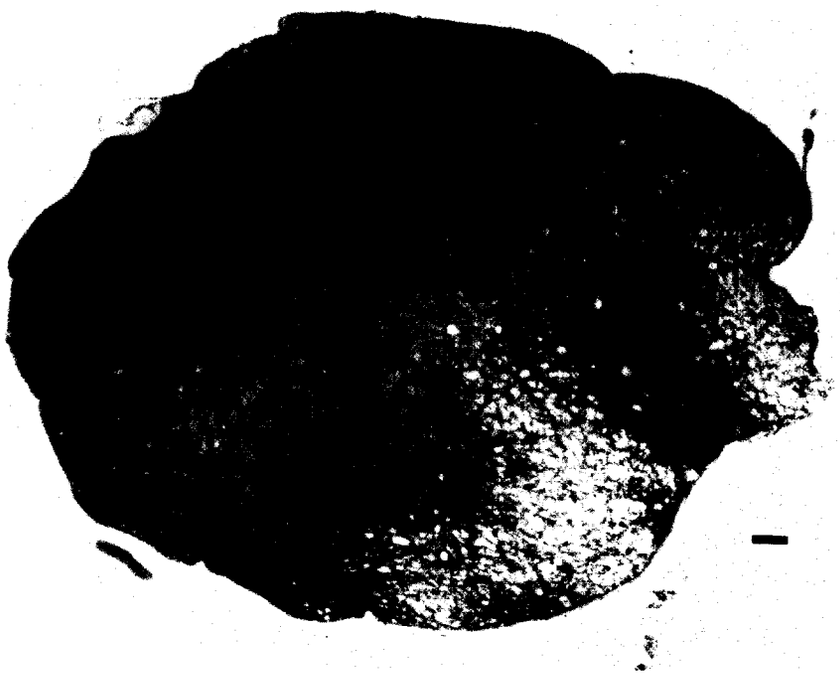

Fig. 1. Light micrograph showing a coronal section of spinal cord of mouse infected 32 days earlier with a demyelinating variant of mouse hepatitis virus (MHV). Two large foci of white-matter demyelination are evident in the ventral funiculi and adjacent to the ventral median fissure. The section is stained with luxol fast blue. Scale bar = 100 μm.

rodents go through progressive stages of subacute, acute and chronic disease accompanied by demyelination and remyelination[5,15,18]; and (3) in terms of neuropathology, MS patients and MHV-infected mice display multifocal white-matter lesions accompanied by myelin stripping[3,5,15,18–20]; in both cases, immune mechanisms are thought to participate in the disease. The cytokines TNF-α (tumor necrosis factor α), IFN-γ (interferon γ) and IL-1 (interleukin 1), as well as the enzyme-inducible nitric oxide synthase (NOS-2), which is responsible for high-level output of the free radical nitric oxide (NO), have all been found to be localized to white matter plaques in humans with MS and MHV-infected mice experiencing demyelinating disease[21–26].

It should be emphasized that, in addition to the murine coronavirus model of demyelination, there are a number of other excellent animal models for MS, such as the Theiler's murine encephalomyelitis virus (TMEV) system and the experimental allergic encephalomyelitis (EAE) model in mice and rats[27]. This brief review will concentrate on the MHV/rodent system and its use as a model for human demyelinating disease.

Background

MHV is an enveloped virus containing a 32-kb single-stranded RNA genome that replicates exclusively in the cytoplasm of infected cells[28]. Virus replication occurs via a viral RNA-dependent RNA polymerase translated from open reading frame one of the genomic RNA. The three predominant structural proteins identified in MHV are the nucleocapsid protein (N; 60 kDa), which is associated with the RNA genome, the membrane protein (M; 25 kDa) and the spike protein (S; 180 kDa)[8,28]. The S protein assembles into a trimeric peplomer, which exhibits a characteristic morphology described as a 'lollipop-like' spike, extending 20 nm from the surface of the virion[4]. In addition, a hemaglutinin-esterase protein (HE; 65 kDa) is expressed by some strains of MHV (Refs 4,28).

The S protein is an important determinant of MHV biology and pathogenesis[4,5,14–16,28,29]. Functions associated with the S protein include binding to host cell receptors, induction of fusion of viral envelope with cell membrane during entry, and induction of cell fusion (syncytium formation)[28]. Williams *et al.*[30] have shown that the S protein recognizes a cellular receptor (MHV-R), which is a mouse biliary glycoprotein (BGP A) and a member of the murine carcinoembryonic antigen (CEA) family of glycoproteins. The MHV-R is found predominantly in the brush border of the small intestine and liver and is nearly undetectable in the brain. Recently, Chen *et al.*[31] have reported a pregnancy-specific glycoprotein member of the CEA family, which is expressed in the brains of C57Bl/6 mice and serves as a receptor for various strains of MHV.

As with many viruses, the outcome of infection with MHV depends upon a variety of different factors, such as host and viral genetics and the dose and route of inoculation. For example, intranasal (i.n.) and intracranial (i.c.) infection of susceptible strains of mice, such as BALB/c and C57Bl/6, with wild-type MHV (MHV-JHM) results in a rapid and fatal encephalomyelitis that is accompanied by gray-matter involvement, with infection of neurons, oligodendrocytes and astrocytes and extensive damage to large areas of the olfactory and limbic system[14,15]. The small percentage of mice that survive the initial bout of acute encephalomyelitis may develop a chronic demyelinating disease that is characterized by extensive white-matter involvement and episodes of demyelination (Fig. 1)[14,15]. The mice may also develop a hindlimb paralysis with some animals even developing tetraplegia. In contrast, infection of SJL/J mice with MHV-JHM does not result in either an acute encephalitis or demyelination. The difference in genetic susceptibility between BALB/c and SJL/J to MHV-JHM is based on polymorphism in the BGP gene and is reflected in the sensitivity of neurons and macrophages to the virus[32,33].

A number of different attentuated strains of MHV have been developed from the wild-type virus (MHV-JHM) through the use of either monoclonal antibodies raised against the S protein[29,34], temperature-sensitive mutants[35] or long-term passage in cell culture[36]. Mice

infected with these neuroattenuated strains generally do not develop fatal encephalitis but may develop neurological disease that is characterized by mononuclear infiltration into the brain and spinal cord, as well as chronic demyelination[14–16,37]. Neuroattentuated strains of MHV tend to spread very slowly through the CNS with little neuronal infection and the majority of infected cells being glia. It has been postulated that the slow rate of spread of certain neuroattenuated strains of MHV allows the intervention of the host immune response and, thus, the elimination of the bulk of infecting virus[37].

Immune response and demyelination

The immune response to MHV infection of the CNS plays a critical role in contributing to the pathogenesis of demyelination. Infection of Lewis rats with MHV-JHM results in the development of acute encephalomyelitis and chronic demyelinating disease. At the onset of clinical symptoms, for example hindlimb paralysis, lymphocytes (both $CD4^+$ and $CD8^+$ T cells) enter the brain[38]. However, when the animals convalesce, the number of infiltrating T cells drops to the levels seen before the occurrence of symptoms, suggesting that the T cells are participating in the disease process[38].

Gamma irradiation of MHV-infected mice results in higher titers of virus compared with non-irradiated control mice, but irradiated animals do not develop demyelination[39]. Adoptive transfer of MHV-immune splenocytes restores demyelination to the infected irradiated mice[39]. Thy-1^+ cells appear to be essential for the restoration of demyelination, indicating a role for T cells in the disease process. These data suggest that MHV-induced demyelination is immunologically mediated.

A recent study by Houtman and Fleming[40] has used several strains of congenitally immunodeficient mice to discriminate between the role of the immune response in the clearance of infectious virus and in the development of demyelinating disease. Intracranial inoculation of a neuroattenuated MHV variant into immunocompetent C57Bl/6 mice results in clearance of infectious virus and robust demyelination. By contrast, virus-infected severe combined immunodeficient (SCID) mice did not clear the virus and did not develop demyelination before dying 12 days postinfection. Demyelination with incomplete clearance of infectious virus was also observed in MHV-JHM-infected nude mice. Adoptive transfer of immune splenocytes from C57Bl/6 mice, but not from nude mice, to SCID mice results in clearance of infectious virus and demyelination.

These studies substantiate an immunological basis for demyelination in MHV-infected mice and, furthermore, suggest that elements of the immune system that are required for demyelination are distinct from those required for clearance of infectious virus. The fact that nude mice were unable to clear virus yet developed demyelinating disease suggests that conventionally educated T cells are not an essential component for demyelination. The authors suggest that the $\gamma\delta$ subset of T cells, natural killer cells, as well as cytokines, may participate in the demyelinating process[40].

It is also important to consider the major histocompatibility complex (MHC) class I and II antigens in demyelination following infection by MHV of resident cells of the CNS. Previous studies involving MHV induction of MHC antigens on glial cells have been controversial. Infection of primary astrocytes results in an increase in class I expression, although this appears to be dependent upon both the MHV strain and the genetic background of the rodent[41–43]. Recent work by Gilmore *et al.*[42] demonstrates that persistent infection of astrocytes by MHV results in an inhibition of class I expression. Sun *et al.*[25] report no expression of class I or II antigen by astrocytes in chronically infected mice experiencing demyelinating disease. Rather, inflammatory macrophage and microglia appear to be the predominant cell type expressing these antigens[25]. The role of MHC class I in demyelination remains complex in light of recent reports indicating that demyelination can occur in MHV-infected mice that lack either stable expression of the MHC class I molecule[40] or functional $CD8^+$ T cells[44].

Expression of MHC class II has been shown to be important in dictating the susceptibility of demyelinating disease to other animal models, such as TMEV (Ref. 45) and EAE (Ref. 46). Furthermore, infection of astrocytes from MHV-susceptible Lewis rats with MHV-JHM results in induction of MHC class II on the surface of the cell, whereas no similar increase was observed in astrocytes from disease-resistant Brown Norway rats[47,48]. However, mice deficient in class II expression express robust demyelination following infection with MHV-JHM (Ref. 40). Therefore, it may be possible that other genes, in addition to, or distinct from, MHC class I and II, may control the outcome of demyelination.

The question of whether MHV infection generates autoimmune T cells, which contribute to demyelination, is controversial and appears to depend on the system being studied. Recent work by Talbot *et al.*[49] has demonstrated the presence of T cells that are cross-reactive with myelin basic protein (MBP) and human coronavirus antigen from patients suffering from MS. Watanabe *et al.*[50] have reported that MBP-specific T cells are generated in rats infected with MHV-JHM. Transfer of these cells to naive animals results in CNS inflammation but not significant demyelinating lesions. The potential for such an autoimmune response against myelin epitopes in the MHV/mouse model must be considered[51].

MHV persistence and demyelination

Recent reports have suggested that human coronavirus RNA may persist in the CNS in demyelinating lesions of patients with MS (Refs 6,7). MHV-infected mice develop chronic demyelinating disease, yet it is not always possible to isolate infectious virus from the CNS compartment. Thus, it is important to consider the nature of viral persistence in the MHV/mouse model.

Recent investigation of the evolution of MHV RNAs during persistence in the CNS has demonstrated that the genomic RNA rapidly evolves into a diverse population of mutant RNA quasi-species within the CNS.

Box 1. Potential mechanisms of demyelination induced by mouse hepatitis virus (MHV)

Virus persistence
- Disrupts oligodendrocyte function
- In astrocytes, results in chronic NOS-2 (nitric oxide synthase), TNF-α (tumor necrosis factor α) and IL-6 (interleukin 6) expression and glial toxicity
- Persistent viral antigens expressed in glial cells target chronic immune response to white matter

Molecular mimicry
- Virus infection primes immune response to crossreact with myelin antigens, such as myelin basic protein (MBP) and proteolipid protein (PLP)

Adami *et al.*[52] investigated coronaviral persistence by examining viral sequences from the brains of mice from 0–42 days postinfection with a neuroattenuated variant of MHV. Although infectious virus was not detected in the brain beyond 13 days postinfection, viral RNA was easily detected by reverse transcription–polymerase chain reaction (RT–PCR) amplification. Sequence analysis of the cloned PCR products revealed that multiple point and deletion mutations had occurred, many of which were concentrated in both the S and N sequences. Greater than 65% of the nucleotide changes observed resulted in amino acid changes, suggesting that a strong selective pressure exists within the CNS. Many of the mutations and deletions reported in the S sequence were concentrated in the hypervariable domain, originally described by Parker *et al.*[53] and continued by Banner and Lai[54]. These findings suggest that a diverse population of RNA quasi-species, many of which are likely to be defective, persist within the CNS. The consequences of this type of infection may contribute to the pathogenesis of chronic demyelination by perturbing normal cellular function or, if the RNAs are translationally active, by chronically stimulating the immune system[53].

Table 2. Host factors that may contribute to virus-induced demyelination

Host factor	Pathology
TNF-α	Recruitment of inflammatory cells to CNS
	Direct toxicity to oligodendrocytes
	Induction of neuronal apoptosis
IFN-γ	T-cell activation
	Induction of the major histocompatibility complex (MHC) and the receptor for the Fc domain of immunoglobulin
	Macrophage/microglial activation
IL-3	T-cell proliferation
IL-4	T-cell proliferation
IL-6	Enhancement of antibody response
	Direct toxicity to oligodendrocytes
NOS-2	Nitric oxide production
	Toxicity to oligodendrocytes

Abbreviations: CNS, central nervous system; IL-3, interleukin 3; IL-4, interleukin 4; IL-6, interleukin 6; IFN-γ, interferon γ; TNF-α, tumor necrosis factor α; NOS-2, nitric oxide synthase.

Infectious virus can be isolated from mice suffering from demyelinating disease if the animals are infected at the suckling stage and nursed by immunized dams[55,56]. An elegant study by Pewe *et al.*[57] demonstrates that virus persisting in the brain and spinal cords of mice suffering from chronic, but not acute, demyelinating disease undergoes changes within the hypervariable region of the S gene, which has previously been defined as a $CD8^+$ T-cell epitope. These data suggest that these viral variants escape from cytotoxic T lymphocyte (CTL) recognition, which then allows for enhanced viral replication within the CNS and the development of clinical disease[57].

Mechanism(s) of chronic demyelinating disease

A variety of different mechanisms have been postulated to contribute to MHV-induced disease (Box 1). As discussed above, the pathological basis for disease depends on a variety of factors, including the system being studied (i.e. mouse versus rat), the age and genetic background of the rodent and the dose and strain of virus being used. Direct lysis of oligodendrocytes (the cells responsible for myelin production and maintenance in the brain) by MHV during the acute phase of infection has been suggested to be responsible for acute demyelinating lesions in the CNS (Refs 19,20,35).

The mechanism(s) responsible for demyelination in chronically infected animals is less understood. Although MHV infects glia, such as astrocytes and oligodendrocytes, readily *in vitro* and *in vivo*, recent work by Perlman and Reis[58] has shown that astrocytes are the cells that harbor the virus within the brains and spinal cords of infected animals suffering from chronic disease. Moreover, histological evidence suggests that MHV-infected astrocytes are activated, as measured by increased staining for glial fibrillary acidic protein (GFAP)[25]. These observations are interesting because astrocytes are closely associated with oligodendrocytes and are required by the oligodendrocyte for normal cellular function. Therefore, any perturbation of astrocyte function and/or production of toxic factors, for example cytokines and NO, may have dramatic effects on the surrounding microenvironment and may contribute potentially to demyelination[25] (Table 2).

Sun *et al.*[25] have measured increased staining for the cytokines TNF-α, IL-1β, and IL-6, as well as NOS-2, in the spinal cords of mice chronically infected with MHV and containing demyelinating lesions. Although associated with areas of viral infection and demyelination, the activated astrocytes were generally not infected with virus. Both NO and TNF-α have been shown to be toxic to oligodendrocytes[59–61]. In the murine EAE model, inhibition of NOS-2 activity ameliorates demyelination, suggesting a contributory role for NO in the demyelinating process[62]. Furthermore, recent work by Boullerne *et al.*[63] suggests that NO may be involved in MS demyelination. Sun *et al.*[25] speculate that production of these factors may contribute

to the demyelinating process. However, there is some controversy as to the role TNF-α has in the disease process; Stohlman *et al.*[64] have reported that administration of antibodies against TNF-α does not ameliorate either MHV-induced encephalomyelitis or demyelination, suggesting that the production of TNF-α alone is not central to the disease process.

In addition to the role of T cells and cytokines in virus clearance and demyelination, our laboratory is focusing on the role played by chemokines in the demyelinating disease process. Chemokines function as chemoattractants for specific populations of cells to sites of inflammation. Recent work has demonstrated a prominent role for the chemokines MIP (macrophage inflammatory protein)-1α, MIP-1β and RANTES (regulated upon activation normal T-expressed and presumably secreted protein) during the acute phase of EAE (Refs 65, 66). Furthermore, glial cells can produce chemokines and potentially contribute to the development of EAE and, possibly, other neurodegenerative diseases[65,66]. It is interesting to speculate that chemokines may be contributing to MHV-induced demyelinating disease by attracting the effector cells, for example monocytes and/or T cells, to the brain and spinal cord, which may then participate in demyelination.

Conclusions

This brief review has focused on the use of the murine coronavirus as a model for human demyelinating diseases and has addressed several unresolved questions in the field of coronavirus-induced demyelination. We have touched on recent advances in the understanding of the mechanism(s) involved in MHV-induced demyelination in rodents. Although exciting epidemiological evidence suggests a viral etiology or cofactor in MS, direct evidence is elusive. With the advent of more-sensitive techniques for virus detection, accompanied by increasing advances in the understanding of the complex immunological mechanisms involved in CNS diseases, a better understanding of human demyelinating diseases lies on the horizon.

Questions for future research

- What is the state of persisting viral RNA in mice undergoing chronic demyelination?
- What role do cytotoxic T lymphocyte (CTL) escape mutants play in chronic demyelinating disease?
- What is the role of autoimmune response in mouse hepatitis virus (MHV)-induced demyelination?
- What are the roles of inflammatory cytokines, chemokines and nitric oxide in the demyelinating process?
- What are the mechanism(s) for remyelination during chronic MHV infection?
- What are the effector mechanism(s) for clearance of MHV from the rodent central nervous system?

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References

1 Oldstone, M.B.A. *et al.* (1996) *Curr. Top. Microbiol. Immunol.* 206, 67–83
2 Allen, I. and Brankin, B. (1993) *J. Neuropathol. Exp. Neurol.* 52, 95–105
3 Steinman, L. (1996) *Cell* 85, 299–302
4 McIntosh, K. (1996) in *Fields Virology* (3rd edn) (Fields, B.N. *et al.*, eds), pp. 401–430, Lippincott-Raven
5 Myint, S.H. (1995) in *The Coronaviridae* (Siddell, S.G., ed.), pp. 389–398, Plenum Press
6 Murray, R.S. *et al.* (1992) *Ann. Neurol.* 31, 525–533
7 Stewart, J.N., Mounir, S. and Talbot, P.J. (1992) *Virology* 191, 502–505
8 Salmi, A. *et al.* (1982) *Neurology* 32, 292–295
9 Murray, R.S. *et al.* (1992) *Virology* 188, 274–284
10 Bergstrom, T., Anderson, O. and Vahlne, A. (1989) *Ann. Neurol.* 26, 283–285
11 Field, E.J. *et al.* (1972) *Lancet* 2, 387–390
12 Koprowski, H. *et al.* (1985) *Nature* 318, 154–160
13 Johnson, R.T. (1994) *Ann. Neurol.* 36, 554–560
14 Dales, S. and Anderson, R. (1995) in *The Coronaviridae* (Siddell, S.G., ed.), pp. 257–282, Plenum Press
15 Fazakerley, J.K. and Buchmeier, M.J. (1993) in *Advances in Virus Research* (Vol. 42) (Maramorosch, K. *et al.*, eds), pp. 249–297, Academic Press
16 Buchmeier, M.J., Dalziel, R.G. and Koolen, M.J.M. (1988) *J. Neuroimmunol.* 20, 111–116
17 Kyuma, S. *et al.* (1992) *Microb. Pathog.* 12, 95–104
18 Raine, C.S. (1994) *Ann. Neurol.* 36, 561–572
19 Lampert, P.W., Sims, J.K. and Kniazeff, A.J. (1973) *Acta Neuropathol.* 24, 76–85
20 Weiner, L.P. (1973) *Arch. Neurol.* 28, 298–303
21 Hofman, F.M. *et al.* (1986) *J. Immunol.* 136, 3239–3245
22 Hofman, F.M. *et al.* (1989) *J. Exp. Med.* 170, 607–612
23 Selmaj, K.W. (1992) *Semin. Neurosci.* 4, 221–229
24 Bö, L. *et al.* (1994) *Ann. Neurol.* 36, 778–786
25 Sun, N. *et al.* (1995) *Virology* 213, 482–493
26 Nathan, C. and Xie, Q. (1994) *Cell* 78, 915–918
27 Tsunoda, I. and Fujinami, R.S. (1996) *J. Neuropathol. Exp. Neurol.* 55, 673–686
28 Holmes, K.V. and Lai, M.M.C. (1996) in *Fields Virology* (3rd edn) (Fields, B.N. *et al.*, eds), pp. 1075–1094, Lippincott-Raven
29 Dalziel, R.G. *et al.* (1986) *J. Virol.* 59, 463–471
30 Williams, R.K. *et al.* (1990) *J. Virol.* 64, 3817–3823
31 Chen, D.S. *et al.* (1995) *Proc. Natl. Acad. Sci. U. S. A.* 92, 12095–12099
32 Knobler, R.L., Haspel, M.V. and Oldstone, M.B.A. (1981) *J. Exp. Med.* 153, 832–843
33 Dveksler, G.S. *et al.* (1993) *Adv. Exp. Med. Biol.* 342, 267–272
34 Fleming, J.O. *et al.* (1986) *J. Virol.* 58, 869–875
35 Haspel, M.V., Lampert, P.W. and Oldstone, M.B.A. (1978) *Proc. Natl. Acad. Sci. U. S. A.* 75, 4033–4036
36 Gallagher, T.M., Escarmis, C. and Buchmeier, M.J. (1991) *J. Virol.* 65, 1916–1928
37 Fazakerley, J.K. *et al.* (1992) *Virology* 187, 178–188
38 Dorries, R. (1994) *J. Neurol. Neurosurg. Psychiatry* 57, 518–520
39 Wang, F-I., Stohlman, S.A. and Fleming, J.O. (1990) *J. Neuroimmunol.* 30, 31–41
40 Houtman, J.J. and Fleming, J.O. (1996) *J. Neurol. Virol.* 2, 101–110
41 Suzumura, A. (1986) *Science* 232, 991–993
42 Gilmore, W., Correale, J. and Weiner, L.P. (1994) *J. Exp. Med.* 180, 1013–1023
43 Joseph, J. *et al.* (1990) *Adv. Exp. Med. Biol.* 276, 579–591
44 Gombold, J.L. *et al.* (1995) *Microb. Pathog.* 18, 211–221
45 Borrow, P. and Nash, A.A. (1992) *Immunology* 76, 133–139
46 Massa, P.T., ter Meulen, V. and Fontana, A. (1987) *Proc. Natl. Acad. Sci. U. S. A.* 84, 4219–4227

47 Massa, P.T., Brinkman, R. and ter Meulen, V. (1987) *J. Exp. Med.* 166, 259–266
48 Massa, P.T. *et al.* (1987) *Adv. Exp. Med. Biol.* 218, 203–211
49 Talbot, P.J. *et al.* (1996) *Ann. Neurol.* 39, 233–240
50 Watanabe, R., Wege, H. and ter Meulen, V. (1983) *Nature* 305, 150–152
51 Jouvenne, P. *et al.* (1992) *Virus Res.* 22, 125–141
52 Adami, C. *et al.* (1995) *Virology* 209, 337–346
53 Parker, S., Gallagher, T. and Buchmeier, M.J. (1989) *Virology* 173, 664–673
54 Banner, L.R. and Lai, M.M. (1991) *Virology* 185, 441–445
55 Perlman, S. *et al.* (1987) *Microb. Pathog.* 2, 185–194
56 Perlman, S. *et al.* (1990) *Virology* 175, 418–426
57 Pewe, L. *et al.* (1996) *Immunity* 5, 253–262
58 Perlman, S. and Reis, A. (1987) *Microb. Pathog.* 3, 309–314
59 Merrill, J.E. *et al.* (1993) *J. Immunol.* 151, 2132–2141
60 Selmaj, K.W. and Raine, C.S. (1988) *Ann. Neurol.* 23, 339–346
61 Selmaj, K.W. *et al.* (1991) *J. Immunol.* 147, 1522–1529
62 Cross, A.H.M. *et al.* (1993) *J. Clin. Invest.* 93, 2684–2690
63 Boullerne, A.I. *et al.* (1995) *J. Neuroimmunol.* 60, 117–124
64 Stohlman, S.A. *et al.* (1995) *J. Virol.* 69, 5898–5903
65 Hayashi, M. *et al.* (1995) *J. Neuroimmunol.* 60, 143–150
66 Godiska, R. *et al.* (1995) *J. Neuroimmunol.* 58, 167–176

Vaccination in pulses: a strategy for global eradication of measles and polio?

D. James Nokes and Jonathan Swinton

Recent American successes against poliomyelitis and measles have been attributed to repeated 'pulse' vaccination campaigns. Whilst logistic and economic constraints will be crucial, a deeper epidemiological understanding of the mechanism, strengths and weaknesses of pulse vaccination will optimize the chances of success elsewhere in the world.

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The global eradication of smallpox, declared in 1979 (Ref. 1), was hoped to be the first in a rapid sequence of successes in the fight to control the major childhood infectious diseases by vaccination. In spite of major achievements in raising global vaccination coverage through routine infant immunization, today it is clear that infections such as measles, poliomyelitis and whooping cough are highly effective adversaries and continue to exact a huge toll in death and disability, particularly in the developing world[2,3].

In the light of greater understanding of their epidemiology and the level of resources and health service infrastructure that are likely to be needed to interrupt transmission, a less-sanguine outlook on our chances of global eradication of these childhood scourges now exists. Theoretical study has identified a level of vaccination coverage (achieving a 'herd immunity' threshold[4,5]) above which persistence of infection would be unlikely. The threshold is agent and population specific but generally falls around 80–95% of the population having vaccine-induced immunity[4]. Smallpox was something of an outlier, with a predicted threshold of nearer 75% (Ref. 6). Achieving these high levels of coverage represents a daunting task and, in the face of financial, political, motivational and communication difficulties, may not be possible through routine primary health services in many parts of the world.

In this context, it is not surprising that the achievements of the past decade in Central and South America have become a focus of attention. In 1994, the Americas were declared polio free; the last case recorded was in Peru in 1991 (Ref. 7). Furthermore, throughout the Americas reports of measles are at an all-time low, with sights firmly set upon regional elimination within the next few years[8]. Much of this success has been attributed to the contribution of vaccination campaigns, repeated at intervals, in which children of a wide age range are offered vaccine[9,10]. Such campaigns, it is reasoned, achieve their effect by rapidly starving the infectious disease of its supply of susceptible individuals[10–12]. In a single campaign, a large fraction of the pool of susceptibles in a population may be immunized. The effect is to decrease incidence drastically. In contrast to this, routine infant immunization procedures only immunize part of each yearly birth cohort; the resultant impact on incidence is slower and less dramatic. In theory, following a campaign or pulse of vaccine, there is no further need to vaccinate until the susceptible fraction has been restored through births to its original or epidemic threshold[13–15].

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