A mouse model of persistent brain infection with recombinant Measles virus

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Measles virus (MV) nucleocapsids are present abundantly in brain cells of patients with subacute sclerosing panencephalitis (SSPE). This invariably lethal brain disease develops years after acute measles as result of a persistent MV infection. Various rodent models for MV infection of the central nervous system (CNS) have been described in the past, in which the detection of viral antigens is based on histological staining procedures of paraffin embedded brains. Here, the usage of a recombinant MV (MV-EGFP-CAMH) expressing the haemagglutinin (H) of the rodent-adapted MV-strain CAM/RB and the enhanced green fluorescent protein (EGFP) is described. In newborn rodents the virus infects neurons and causes an acute lethal encephalitis. From 2 weeks on, when the immune system of the genetically unmodified animal is maturing, intracerebral (i.c.) infection is overcome subclinically, however, a focal persistent infection in groups of neurons remains. The complete brain can be analysed in 50 or 100 μm slices, and infected autofluorescent cells are readily detected. Seven and 28 days post-infection (p.i.) 86 and 81 % of mice are infected, respectively, and virus persists for more than 50 days p.i. Intraperitoneal immunization with MV 1 week before infection, but not after infection, protects and prevents persistence. The high percentage of persistence demonstrates that this is a reliable and useful model of a persistent CNS infection in fully immunocompetent mice, which allows the investigation of determinants of the immune system.

INTRODUCTION

The human progressive neurodegenerative brain disease subacute sclerosing panencephalitis (SSPE) is caused by a persistent measles virus (MV) infection, which becomes clinically apparent years after the acute infection. According to new data (Bellini et al., 2005) approximately one in 10 000 children infected with wild-type MV, most of which were younger than 2 years at the time of infection, develop SSPE after about 6–10 years. It is interesting that wild-type and not vaccine virus induces the disease, and it is unknown why wild-type virus switches its clinical manifestation from an acute disease to a chronic and invariably fatal CNS infection. In the brains of SSPE patients, defective hypermutated MV is detected in neurons, glial cells and endothelial cells, and spreads predominantly from cell to cell as a ribonucleoprotein particle (RNP) in the absence of free infectious virus (Baczko et al., 1993). If at all, infectious virus can be isolated from recent lesions localized with magnetic resonance imaging (Ogura et al., 1997). Chronic infection of the brain proceeds until death of the patient in spite of a presumably normal cellular immune response and generation of high levels of intrathecal antiviral antibodies. A specific treatment of the disease does not exist (ter Meulen et al., 1983; Cattaneo et al., 1988; Norrby & Kristensson, 1997; Griffin, 2001; Weissbrich et al., 2003).

Several animal models for MV-induced brain diseases using hamster, rat and mice have been described in the past (Liebert & Finke, 1995). In these models either rodent brain-adapted MV strains (HNT or CAM/RB) were used in genetically unmodified animals or the attenuated MV strain Edmonston (MV-Edm) was used in transgenic mice, which expresses an MV receptor. Whereas intracerebral (i.c.) infection of newborn mice leads to a lethal encephalitis in all mice, even expression of the cellular receptors for MV (CD46 and CD150) in adult transgenic mice is not sufficient to elicit a disease. This was only possible if transgenic expression of MV receptors was combined with immunodeficiencies (Horvat et al., 1996; Niewiesk et al., 1997; Thorley et al., 1997; Blixenkrone-Moller et al., 1998; Mrkic et al., 1998; Lawrence et al., 1999; Evlashev et al., 2000;
Patterson et al., 2001a; Oldstone et al., 2005; Shingai et al., 2005; Welstead et al., 2005). In contrast, the rodent-adapted MV-strain CAM/RB induces an acute encephalitis in genetically unmodified susceptible mice, which differ in the efficacy of their immune response from resistant mouse strains (Niewiesk et al., 1993). In terms of the immune response, the ability to mount an efficient CD4 T-cell response with interferon gamma (IFN-γ) secretion has been demonstrated to be crucial for protection (Finke & Liebert, 1994; Finke et al., 1995; Weidinger et al., 2000). The elimination of virus could either be due to effective mouse strain-specific antigen presentation (Weidinger et al., 2001) or to an induced intracellular antiviral activity, which presents as a non-cytolytic mechanism (Patterson et al., 2002).

So far, the only model for studying chronic MV infection in the central nervous system (CNS) of immunocompetent mice is the inoculation of neonatal mice with MV-Edm, which leads to a persistent infection in BALB/c mice for about 21 days. Thereafter the animals start clearing the virus, which correlated with an increase of the MV-specific CD4 T-cell response (Calsillo et al., 2004). In this model virus load was determined by real-time PCR, which is more sensitive than virus isolation, but does not allow the localization of actively replicating virus (Calsillo et al., 2004). Our past studies have shown that these limitations might be overcome with recombinant MV-expressing enhanced green fluorescent protein (EGFP) in order to visualize infected cells and to indicate active replication in vitro and in vivo (Duprex et al., 2000). We have also demonstrated that the haemagglutinin (H) is an important determinant of viral pathogenicity. When the haemagglutinin of CAM/RB was introduced into the molecular clone of the attenuated MV-Edm, this virus became lethal in normal mice (Duprex et al., 1999b; Moeller et al., 2001). In this study, we describe the utilization of a mouse-pathogenic recombinant MV (MV-EGFP-CAMH) expressing the rodent-adapted CAM-H protein and EGFP in order to infect mice at various ages to induce a persistent chronic brain infection.

**METHODS**

*Viruses and cells.* The recombinant virus MV-EGFP-CAMH was rescued from a modified full-length construct following Lipofectin-mediated transfection of MVA-T7-infected HeLa cells as described previously (Duprex et al., 1999a, b, 2000). The growth characteristics of the MV-EGFP-CAMH was compared to EdtagCAMH, which does not contain the additional transcription unit from which EGFP is expressed, and no significant effect on replication or final titres was observed (Ludlow, 2003).

For virus production, Vero cells were infected at an m.o.i. of 0-01 and virus was harvested when maximum giant cell formation was observed by one cycle of freezing–thawing and two times pelleting cell debris by centrifugation. Viral stocks were stored at −80 °C.

*Animal infection, histology and antibodies.* Specific-pathogen-free animals were purchased from Harlan-Winkelmann. Mice (C57BL/6 and BALB/c) were infected i.c. into the left hemisphere with 20 μl virus suspension containing 1 × 10^7 p.f.u. or as given in the text. For analyses, animals were anaesthetized, blood was taken to prepare serum and then animals were perfused with 4 % (w/v) paraformaldehyde (PFA). Brains were fixed in 4 % PFA for at least 18 h, and free floating sections (50 or 100 μm) were prepared using a vibratome (Technical Products International). Slices were analysed directly or incubated with blocking buffer containing 10 % BSA and 2:5 × Triton X-100 in PBS, washed in PBS containing 1 % BSA and 0:25 % Triton X-100, incubated with primary antibodies in washing buffer in 48-well plates overnight at 4 °C, washed, incubated with fluorescent dye-conjugated secondary antibodies, washed and analysed by UV microscopy. Monoclonal phycoerythrin (PE)-conjugated anti-mouse CD4- and CD8-antibodies were purchased from Becton Dickinson. Secondary antibodies were peroxidase-conjugated goat anti-mouse (Dianova). Photomicrographs were taken with a digital camera (Leica). Statistical analyses with the Student’s t test were done using the program Prism (GraphPad).

**Determination of anti-MV immunoglobulins by ELISA.** Sera were taken from mice when they were sacrificed and the anti-MV titres determined by ELISA. Briefly, 96-well plates (Nunc) were coated overnight with purified MV (1 μg per well) in coating buffer (50 mM Na2CO3, 0:02 % Na2SO4, pH 9:5). Plates were washed with 350 mM NaCl, 0-1 % Tween 20, 50 mM Tris pH 7-5. Serial dilutions of sera were added in 350 mM NaCl, 0-1 % Tween 20, 50 mM Tris pH 7-5 containing 1 % BSA at 37 °C for 1 h. The wells were incubated with goat anti-mouse peroxidase-conjugated antibodies for 1 h at 37 °C and bound antibodies detected using ortho-phenyl-diamine in citrate buffer and 0-01 % H2O2. The reaction was stopped by adding 0-5 M sulphuric acid. Absorbance was measured at 490 nm on a Bio-Rad plate reader.

**Virus reisolation.** Brains were taken from non-perfused mice, pressed through a steel sieve and homogenized in one additional volume PBS. A 10 % solution in PBS and 1:2 dilutions were overlaid onto Vero cells grown to 30 % confluency, washed three times with PBS containing 0-1 % fetal calf serum after 24 h and incubated for 7 days. Autofluorescent infectious centres were detected by UV microscopy.

**RESULTS**

**Age-dependent survival of i.c.-infected mice**

It has previously been shown that there is an age-dependent susceptibility to i.c. infection with various viruses (Griffin, 1976; Liebert & ter Meulen, 1987; Lawrence et al., 1999). Rodent-adapted MV strains replicate in brains of newborn animals and induce fatal acute measles encephalitis, whereas between weeks 1 and 4 the immune system matures and mice become increasingly resistant, depending on the strain of mouse. We therefore investigated whether a similar age dependency is observed with the new recombinant virus. C57BL/6 and BALB/c mice were infected i.c. with 1 × 10^7 p.f.u. MV-EGFP-CAMH at the age of 1, 7, 14 and 21 days. All neonatally infected mice died after 3–5 days, whereas 20 % of the 7-day-old mice and 95 % of the 14-day-old mice survived (C57BL/6 mice Fig. 1; BALB/c mice not shown). Older mice survived the i.c. infection with no obvious clinical signs of infection. These results suggest that the increasing resistance of the mice to infection with this recombinant virus parallels the maturation of the immune system as described earlier for other viruses.
Detection of infected neurons in subacute and persistently infected brains

In brains of 1- and 2-week-old C57BL/6 mice infected i.c. in the left hemisphere with MV-EGFP-CAMH, cortical groups of autofluorescent neurons were detected in the ipsi- and contralateral hemispheres, demonstrating the transport of infectious virus along neuronal processes. Long processes in the corpus callosum connecting different areas in the brain were also strongly EGFP-positive. The cortical and subcortical (caudal putamen) distribution of infected neurons in a mouse brain infected at 1 week for 7 days was observed in coronal sections (Fig. 2a). A focus of EGFP-positive cortical neurons with fluorescent processes is shown in the enlargement (Fig. 2b). In brains from mice infected at 2 weeks of age, the distribution of infected neurons at 7 days p.i. is restricted more to the cortical neuronal layers and a small number of positive cells is detected subcortically (Fig. 2c). Enlargements are shown in Fig. 2(d), (e) and (f) [arrows 1, 2 and 3, respectively, in (c)]. An example of a brain (left hemisphere) with only one EGFP-positive neuron from a mouse infected at 2 weeks and analysed at 21 days p.i. is shown in Fig. 2(g) and (h). The strong autofluorescence of infected cells easily allows the detection of this single infected cell. Foci and single EGFP-positive neurons were detected for more than 50 days p.i. A single positive neuron at 49 days p.i. is presented in Fig. 2(i). From brains of mice infected at 2 weeks, approximately $10^5$ and $10^6$ p.f.u. per brain infectious MV was reisolated at 7 and 14 days p.i. Thereafter only very little or no infectious virus could be isolated from single randomly selected brains.

Statistical analysis of virus persistence

Fixed brains were sectioned from the olfactory bulb to the cerebellum into approximately 30–40 coronal sections of 50 or 100 μm thickness, depending on the age of the animals, allowing the complete brain to be examined for EGFP-positive cells. Two-week-old mice were infected i.c. and analysed 7 (at day 21 after birth) and 28 days p.i. (at day 42 after birth). At 7 days p.i., autofluorescence was observed in 86% of left and 71% of right hemispheres (Table 1). Interestingly, nearly the same percentages (81 and 69%, respectively) of MV-positive brains were obtained 28 days p.i. The differences between right and left hemispheres, and 7 and 28 days p.i., are not significant. The number of positive neurons per brain at 7 days p.i. was on average $836 \pm 255$ (mean $\pm$ SEM, $n=21$), whereas the number of positive cells detected at 28 days p.i. per brain varied from 0 to 74, with a mean of $15 \pm 3.7$ ($n=26$). Thus, the highly sensitive detection of MV-EGFP-CAMH and the high percentage of persistently infected brains enable the analysis of factors influencing the establishment and maintenance of the persistent infection in fully immunocompetent mice.

Immunization before and after brain infection

We assessed the effect of immunization before and after i.c. infection to identify factors influencing the establishment and/or elimination of this persistent infection. Mice were immunized intraperitoneally (i.p.) with $10^6$ p.f.u. of MV-Edm either at day 7 ($i7$ = 1 week before) or at day 28 ($i28$ = 2 weeks after i.c. infection with MV-EGFP-CAMH), and analysed at day 21 ($7$ days p.i.) and at day 42 (28 days p.i.). The time schedule is given in Fig. 3(a). Replicating recombinant virus was detected only in $29$ (7 days p.i.) and 22% (28 days p.i.) of the mice immunized at day 7 (Table 2). Thus, more than 2/3 of the mice were completely protected by immunization. The difference in the percentage of immunized in comparison to non-immunized mice is highly significant (***, $P \leq 0.0003$). In addition, the number of infected cells in brains of pre-immunized mice at 7 days p.i. was drastically reduced in comparison to non-immunized mice ($23 \pm 12$, $n=18$, in comparison to $836 \pm 255$, $n=21$); **, $P=0.0057$). At 28 days p.i., the number of positive neurons in immunized mice was also reduced (on average $2.4 \pm 1.7$, $n=9$) in comparison to non-immunized mice ($15 \pm 3.7$, $n=26$, statistically not significant). Thus, immunization 1 week before infection significantly reduces percentages of infected brains and also the number of infected neurons per brain.

In contrast to immunization before infection, in mice immunized 2 weeks after brain infection, no effect of the immunization on the persistent infection in the brain and the number of infected neurons was detected. In this case 81% of mice had EGFP-positive neurons in the brain at day 42 (= 28 days p.i.) (Table 2), the same percentage as found in non-immunized mice (Table 1). The mean number of infected neurons per brain in mice immunized after infection was even higher, $31 \pm 6.7$ ($n=16$), in comparison to non-immunized mice, $15 \pm 3.7$ ($n=26$; *, $P=0.03$). The data given in Tables 1 and 2 of the left and right hemispheres were taken together and are presented in Fig. 3(b).

Antibody titres obtained from sera of the corresponding mice revealed that both, early and late, immunization led to an enhancement of the humoral anti-MV response (Fig. 3c). Similar antibody titres were measured in mice immunized at
day 7 and at day 28 after 28 days p.i. (Fig. 3c, lanes 4 and 5). In spite of this, the elevated antibody level induced by immunization after brain infection (i28-28 dpi) did not reduce the percentage of infected brains or number of infected cells per brain (Fig. 3b).

**Infiltrating T cells in coronal sections of infected mouse brains**

In order to analyse whether infiltrating cells are present in infected brain areas in our model, and whether the presence of such cells correlates with the level of infection, brains were stained with antibodies recognizing CD4 and CD8, which were directly labelled with red fluorescent dyes. Two-week-old animals were infected with or without prior immunization and brains were analysed 5 and 10 days p.i. (Fig. 4). In non-immunized mice, a considerable number of CD4- and CD8-positive T cells were detected at 5 and 10 days p.i. (Fig. 4a–d). T cells were detected in and around foci of infected neurons in the cortex and surrounding the external capsule and hippocampus. In contrast, in immunized mice already at 5 days p.i. both fewer infected neurons and fewer infiltrating T cells than in non-immunized mice were detected (Fig. 4e and f). At 10 days p.i., almost no infected cells and infiltrating CD4- and CD8-positive cells were detected in immunized mice (Fig. 4g and h). This

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**Fig. 2.** Autofluorescent cells in coronal sections of C57BL/6 mice. Section of a brain from a mouse infected at 1 week, for 7 days p.i. (a), and enlargement of a group of GFP-positive neurons (b, arrow in a). Section of a brain from a mouse infected at 2 weeks, for 7 days p.i. (c), and enlargements as designated by arrows (d, e, f). Section of a brain from a mouse infected at 2 weeks, for 21 days p.i. (g), and enlargement of a single GFP-positive neuron [h, arrow in (g)]. A single GFP-positive neuron 49 days p.i. in the brain of a mouse infected at 2 weeks (i). The approximate localization of the coronal sections shown (j). Magnification for (a), (c) and (g) is ×6; for (b), (d), (e) and (f) is ×40; and for (h) and (i) is ×160.
indicates a rapid and efficient intrathecal elimination of infecting virus from the CNS of immunized mice within the first days of infection.

**DISCUSSION**

Since the development of techniques to generate recombinant measles viruses in the 1990s (Radecke et al., 1995) the resulting viruses have been extensively used in the study of viral immunology and pathogenesis, and has opened a fundamentally different way of studying viral persistence. We have taken advantage of the MV-EGFP-CAMH that allows us to combine highly sensitive detection of EGFP with the neurotropic properties of the rodent-adapted attachment protein of the CAM/RB strain of MV (Duprex et al., 1999b; Moeller et al., 2001). Infection of 2-week-old adolescent mice resulted in a subacute and persistent brain infection that enabled us to set up a novel model of MV persistence in fully immunocompetent mice.

Since the additional transcription unit expressing EGFP is incorporated in the viral genome, the reporter protein is exclusively expressed when viral RNA-dependent RNA polymerase is active and the virus replicates. Furthermore, a high expression level of EGFP is obtained as the additional transcription unit is present in the promoter proximal position and as such the first gene expressed during virus transcription. Cellular factors can influence the EGFP expression only if they influence virus replication in general, and when virus replication stops, EGFP is not produced further. Since the half-life of EGFP in neurons has not been determined, we do not know how long EGFP is detected after termination of virus replication. However, since regions that had been highly positive in brains at 7 days p.i., lack EGFP-positivity later, and there is an almost constant number of strongly positive neurons in persistently infected brains from 21 to 50 days p.i., it appears probable that GFP is completely degraded within a few days. A background type of weak EGFP-positivity was detected in supposedly healed areas of former virus replication in some sections, which can

| Table 1. Percentage of infected cells detected in brains of acutely and persistently infected C57BL/6 mice |
|---|---|---|---|---|
| Expt no. | No. animals | Age of mice at i.c. infection (days) | Analysis at day | No. MV+/no. tested (left and right hemisphere) |
| 1 | 7 | 14 | 21* | 5/7 5/7 |
| 2 | 8 | 15 | 21 | 7/8 5/8 |
| 3 | 6 | 14 | 21 | 6/6 5/6 |
| | | | Sum: 18/21 (86%) | 15/21 (71%) |
| 4 | 6 | 14 | 42† | 4/6 4/6 |
| 5 | 7 | 13 | 42 | 6/7 6/7 |
| 6 | 5 | 14 | 42 | 4/5 3/5 |
| 7 | 8 | 14 | 42 | 7/8 5/8 |
| | | | Sum: 21/26 (81%) | 18/26 (69%) |

*Day 21 = 7 days p.i.
†Day 42 = 28 days p.i.

| Table 2. Influence of immunization before and after i.c. infection on acute and persistent brain infections |
|---|---|---|---|---|---|
| Expt no. | No. animals | Immunization at day | Age of mice at i.c. infection (days) | Immunization at day | Analysis at day | No. MV+/no. tested (left and right hemisphere) |
| 1 | 9 | 7 | 14 | – | 21* | 3/9 2/9 |
| 2 | 8 | 7 | 14 | – | 21 | 2/8 1/8 |
| | | | | | Sum: 5/17 (29%) | 3/17 (18%) |
| 3 | 9 | 7 | 14 | – | 42† | 2/9 2/9 |
| | | | | | (22%) (22%) |
| 4 | 9 | – | 14 | 28 | 42 | 8/9 7/9 |
| 5 | 7 | – | 14 | 28 | 42 | 5/7 5/7 |
| | | | | | Sum: 13/16 (81%) | 12/16 (75%) |

*Day 21 = 7 days p.i.
†Day 42 = 28 days p.i.
be distinguished clearly from strongly positive neurons with ongoing virus replication. Thus, strong EGFP-positivity after several weeks suggest ongoing virus replication in these neurons. In addition, our data of virus reisolation support these findings.

Infected neurons with EGFP-positive processes were found typically for the MV infection in the cortex, hippocampus, thalamus and brain stem. Not only the left hemisphere, which was i.c. infected, is affected, but infection spreads very efficiently also to the right hemisphere to corresponding neuronal foci. Little is known about mechanisms of virus spread in the brain. An unidirectional retrograde spread of MV along the neuronal processes connecting CA1, CA3 and the dentate gyrus was observed in rat hippocampal slice cultures (Ehrengruber et al., 2002). Two phenomena of the brain of rodents or neuronal cell cultures have been described by several authors: (i) that a receptor is required to enable the initial brain or neuronal infection, and (ii) that further cell-to-cell spread occurs without the requirement of known virus receptors (Meissner & Koschel, 1995; Allen et al., 1996; Rall et al., 1997; Urbanska et al., 1997; McQuaid et al., 1998; Mrkic et al., 1998; Duprex et al., 1999a, 2000; Evlashev et al., 2000; Lawrence et al., 2000; Ehrengruber et al., 2002). The initial requirement for a specific receptor is supported by findings in the transgenic and non-transgenic mouse models. Results obtained with CD46-transgenic animals and MV-Edm proved that the presence of CD46 on neurons enables infection with vaccine-like MVs. Vice versa, data obtained with normal mice and MV with the neurotropic rodent-adapted H protein (CAM-H) suggested that a certain structure of the H protein can interact with an endogenous rodent molecule enabling infection of neurons, since the adaptation to growth in rodent brains is reflected by 11 aa differences between the Edm-H and CAM-H proteins, and the exchange of 2 aa (at positions 195 and 200) in the CAM-H protein is sufficient to omit its capacity to spread in rodent brains (Moeller et al., 2001); it is likely that the subsequent cell-to-cell spread of infectious RNP particles does not require these receptors.

The age dependency of the extent of the CNS infection has been demonstrated earlier, and neonatal MV-receptor transgenic or non-transgenic mice or rats all suffer from acute encephalitis and die within few days depending on the dose of virus (Griffin, 1976; Liebert & ter Meulen, 1987; Lawrence et al., 1999). It is known that newborn animals are deficient in both T helper and cytotoxic T-cell-mediated

Fig. 3. Experimental schedule, summary infection data and anti-MV antibody levels induced in infected mice. (a) The sequence of experimental manipulations is given in dependency of the age of mice (weeks). Mice were infected at day 14, immunization was done either at day 7 (i7) or at day 28 (i28), and microscopical analysis was done 7 and 28 days p.i. (b) The percentages of EGFP-positive brains from separate experiments (left and right hemispheres taken together) from non-immunized (open symbols) and immunized (closed symbols) mice with probability calculations are presented. Lane 1: mice infected at day 14 and analysed 7 days p.i. (7dpi); lane 2: mice immunized at day 7, infected at day 14 and analysed at 7 days p.i. (i7-7dpi); lane 3: mice infected at day 14 and analysed at 28 days p.i. (28dpi); lane 4: mice immunized at day 7, infected at day 14 and analysed at 28 days p.i. (i7-28dpi); and lane 5: mice immunized at day 28, infected at day 14 and analysed at 28 days p.i. (i28-28dpi). (c) Mice were immunized with 10⁶ p.f.u. MV, infected at 2 weeks of age and serum taken 7 or 28 days p.i. The MV-specific titre was determined by ELISA. Individual titres of non-immunized (open symbols) and immunized (closed symbols) mice, and mean titres (n=8, line) are given. The negative control (lane 6) was taken from 6-week-old non-immunized, non-infected mice.
immune responses, which are associated with a low interleukin (IL)-2 and high IL-4 production and deficits in utilization of the cytokines and accessory cell functions (Adkins et al., 1994). Besides factors of the immune system also the susceptibility of neurons may be increased in neonatal mice due to incomplete myelination, which acts as a mechanical barrier against infection. Molecules used as receptors for rodent-adapted strains, as the human receptors in transgenic
mice, appear to be present on neurons in neonatal and adult mice, and depletion of the T-cell response in adult mice results in acute encephalitis (Weidinger et al., 2000). In addition to the T-cell response, also other than lymphocyte-mediated protection mechanisms were observed to delay disease progression in adult animals. These may be components of the innate immune system such as natural killer cell cytotoxicity or inflammatory cytokine secretion (Lawrence et al., 1999). We selected adolescent mice for our experiments because from the age of 14 days on, practically all mice survived the i.c. infection with 10^3 p.f.u. MV-EGFP-CAMH. After widespread intrathecal replication of the virus within the first week, the number of EGFP-positive cells decreases and the remaining foci of actively infected neurons persist for several weeks in a high percentage of brains. These data are a reliable basis for studies of factors influencing the CNS infection. Interestingly, i.p. immunization as early as 7 days after birth protected the majority of mice from virus propagation and persistent infection, whereas immunization after the infection had no effect on the persistent brain infection in spite of high anti-maesles antibody titres. This resembles the situation in human SSPE, where extremely high anti-maesles antibody titres in serum and cerebrospinal fluid are pathognomonic for the disease (Weissbrich et al., 2003). In SSPE, as in the mouse model, the high antibody titres cannot prevent the viral spread in the CNS. This could also be an indication for an early CNS infection in the human before the induction of antiviral antibodies.

CD46 transgenic mice that are unable to mount an adaptive immune response (RAG1−/−) develop a progressive CNS disease when infected with the Edmonston vaccine strain (Lawrence et al., 1999), whereas the course of the disease is more protracted when the animals are infected with a recombinant MV containing the matrix gene of the Biken SSPE isolate (Patterson et al., 2001b). Sequencing of MV-matrix gene from brains of infected immunodeficient mice revealed the presence of mutations and hypermutations (Oldstone et al., 2005) similar to those found in autopsy samples obtained from SSPE patients. In addition, Oldstone et al. (2005) described that transient immunosuppression by a second virus (lymphocytic choriomeningitis virus CI 13) can support the establishment of a non-progressive, persistent CNS infection. In contrast to these findings, which are based on permanent or transient immunodeficiencies, the model of MV persistence we have established utilizes immunocompetent mice and should allow us to examine factors of the immune system influencing the development of the CNS infection.

It is known that the susceptibility or resistance of mice for MV depends on the major histocompatibility complex haplotype and a functional CD4- and CD8-positive T-cell response (Niewiesk et al., 1993; Weidinger et al., 2000). MV infection induces the synthesis of chemokines such as IFN-γ-inducible protein 10 kDa (IP-10) and RANTES in infected neurons maximally between day 7 and 12 after infection of adult CD46-transgenic mice with MV-Edm (Patterson et al., 2003). The protective mechanisms contributing to the rapid virus clearance after early immunization can now be studied in this model.

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