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Subacute Sclerosing Panencephalitis and Other Lethal Encephalitis Caused by Measles Virus Infection: Pathogenesis and New Approaches to Treatment

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1. Introduction

Measles virus (MV) is a human, negative-stranded RNA virus, member of the Paramyxoviridae family, genus Morbillivirus. The virus enters cells by interaction of viral glycoprotein Hemagglutinin (H) with cellular receptors (CD46, CD150, CD147/EMMPRIN) and membrane fusion is mediated by viral fusion glycoprotein (F); helical nucleocapsids replicate in the cytoplasm on replication-transcription complexes formed by the viral catalytic subunit (L), the phosphoprotein (P) and the RNA wrapped in the viral nucleoprotein (N); virus particles bud out from plasmatic cell membrane patches internally lined by viral matrix protein (M). MV causes cytopathic effects by cell fusion forming syncytia, by inducing apoptosis, or both together, and may produce persistent infections in cultured cells and in the infected host. MV is highly lymphotropic infecting macrophages, lymphocytes and dendritic cells; causes systemic acute infections after cell-associated viremia generating life-long immunity (Griffin, 2007 for a review).

Despite the availability of an efficient live attenuated vaccine, MV still remains an important global pathogen infecting over 25 millions individuals and causing over 250.000 deaths per year, being one of the main causes of child death worldwide. Plans for the global eradication of measles are hindered by a number of factors: 1. high contagiousness of MeV (it is the most transmissible respiratory virus known, and it is needed a 95% to 98% protection in a population to avoid measles out-brakes), 2. vaccination fails in over 5% of the general population (non-responders), 3. vaccination has a low efficiency in infants under 9 months, 4. poor health care in some countries, and 5. objection to vaccination in sectors of the population.

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During acute measles, MV produces a transient clinical significant immunosuppression that can contribute to some complications as measles interstitial pneumonitis and giant cell pneumonia, otitis media and diarrhoea. Unfrequently, MV may cause Central Nervous System lethal complications as Acute measles post-infection disseminated encephalomyelitis (ADEM), Measles inclusion body encephalitis (MIBE), and Subacute sclerosing panencephalitis (SSPE) Figure 1 and Table1. In this chapter we will briefly review the epidemiology, clinical course, pathogenesis, treatment, and prevention of these encephalitis with emphasis on SSPE, and present some results from our group concerning pathogenesis and possible therapeutics approaches to this fatal disease.

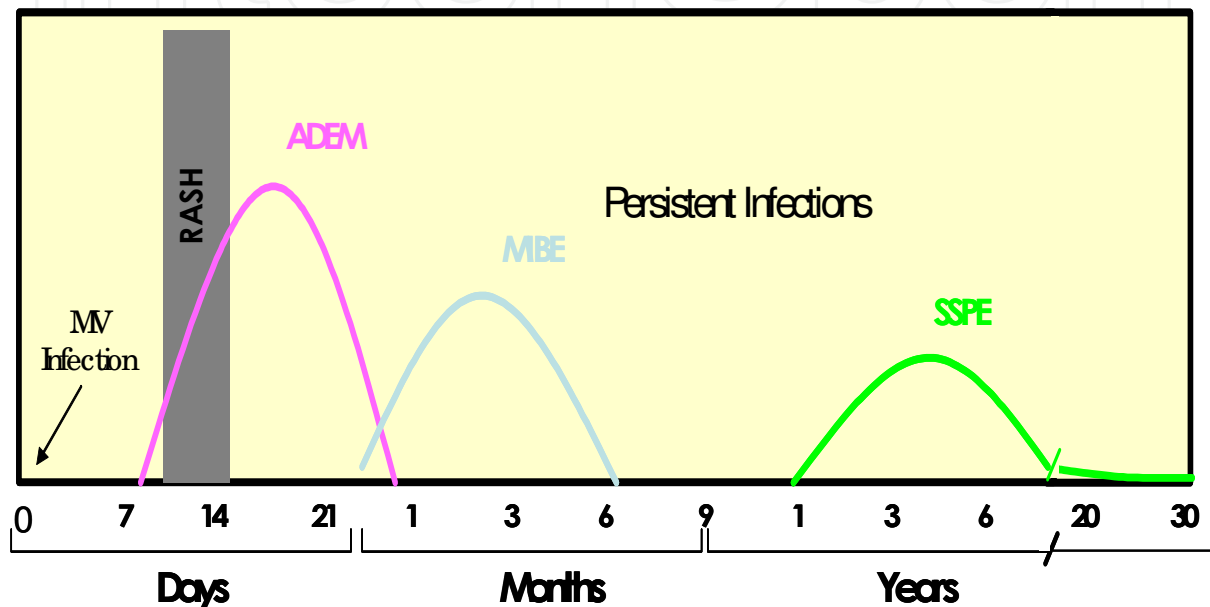


Fig. 1. Neurological complications of Measles Virus Infections. Onset and time course of encephalitis after MV infection (ADEM, MIBE, and SSPE)

2. Acute post-infectious measles disseminated encephalomyelitis (ADEM)

Onset occurs about one to 2 weeks after the appearance of the rash (in some rare cases, coincident with rash) in approximately one case in 10³ cases of measles, usually in children older than 2 years and adults. In contrast, the incidence drops to one in one million after measles vaccination. The onset is typically abrupt, starting with irritability fever, headache, vomiting, and confusion and progressing rapidly to seizures impaired consciousness and coma, Present a monophasic clinical course over weeks and the mortality rate is 10 to 20%. The majority of survivors have neurological sequelae, in one quarter of them permanent. Neither MeV virus, or viral RNA has been found in the brain of patients with ADEM at autopsy, and not intrathecal synthesis of anti-MV antibodies have been demonstrated. Among other pathology changes, perivascular inflammation and demyelination are observed. Possibly it is an autoimmune parainfectious disease. Molecular mimicry between myelin basic protein and MV proteins has been conjectured. Antibodies to myelin basic protein are found in CSF, but no cross-reacting antibodies or T cells have been identified. Besides supportive therapy, immunomodulatory treatment with intravenous (i.v.) corticosteroids, i.v. immunoglobulin or plasmapheresis have been employed in monitored patients with variable results. Current live measles virus vaccine has reduced the incidence of ADEM after vaccination campaigns.

| Disease | Host | Virus | Epidemiology | Onset, and Course | Clinical features | Pathogenesis | Treatment | Prevention |
|--|---|---|---|--|---|---|--|--|
| ADEM Acute Disseminated Encephalomyelitis | Otherwise healthy. Typically older than 2 years. | Wild-type or Vaccinal MV. | Incidence: Approximately 1/10 ³ cases of measles, and 1/10 ⁶ MV vaccinations. | Onset within the first week of rash. Monophasic over weeks. Mortality rate is 10 to 20%. Majority of survivors with neurological sequelae. | Abrupt onset with headache, vomiting, irritability, confusion, frequently recurrence of fever, and seizures. Progressing rapidly to obtundation and coma. | No MV particles, RNA, or proteins detected in the brain, or intrathecal synthesis of MV antibodies. Possibly an acute parainfectious encephalomyelitis without evidence of MV brain invasion. Perivascular inflammation and demyelination. | Besides supportive therapy, iv corticosteroids, iv. immuno globulin and plasmapheresis have been used with variable results. | Current live attenuated MV vaccine has reduced the incidence of ADEM. |
| MIBE Measles Inclusion Body Encephalitis | Immu- deficient patients of any age. (with deficient cell- mediated immunity). | Wild-type MV. Vaccinal MV in severely immuno- deficient infants. | Patients with congenital or acquired immuno- deficiency after exposure to MV. | Onset typically months after infection or exposure to MV. Progressive course over months. Mortality rate is 75 to 85%. | Patients usually present with afebrile refractory focal seizures and altered mentation and progress to generalized seizures, coma, and death. | Brain biopsy or autopsy show gliosis, focal necrosis, perivascular cuffing, in neurons and glia nuclear and cytoplasmic MV inclusion bodies, MV RNA and nucleocapsids. In general no infectious MV virus can be isolated from brain. No detectable anti-MV immune response. | No effective specific treatment exist for MIBE. Early iv. ribavirin could improve symptoms. There is not evidence of IFN-alfa benefit. | Routine MV vaccination of all children may be the best available strategy to reduce the incidence of MIBE. |

| Disease | Host | Virus | Epidemiology | Onset, and Course | Clinical features | Pathogenesis | Treatment | Prevention |
|---|--|---|---|--|---|--|--|---|
| SSPE Subacute Sclerosing Panencephalitis | Otherwise healthy individuals with previous infection or exposure to MV, in the majority of cases under 2 years of age. Genetic pre-disposition, and environmental factors, as coincident infection by other pathogens, might be involved. | Wild-type MV belonging to different genotypes. There is no evidence of MV vaccine causing SSPE. | SSPE incidence is 4 to 11 per 10 ⁵ cases of measles (18 per 10 ⁵ , when measles at age under 2 years) The incidence of SSPE has declined dramatically years after the introduction of MV vaccination programmes. SSPE predominates in males at approximately a 2.5 ratio. Very rarely, small clusters of SSPE has been described. SSPE development in only one of identical twins after coincident measles. | Onset on the average 8 years after MV acute infection. The incubation period ranging from one year to several decades after MV acute infection. The course is progressive from one to 20 years (some times with transient remission periods), but in most cases death occur within 3 years of onset. | Insidious onset with symptoms of progressive cortical dysfunction (deterioration of intellectual capacity, and some times awkwardness, stumbling and symptoms of retinitis). Later, motor disability and paroxysmal disorders develop: myoclonic jerks, convulsive seizures and ataxia. Next stage is characterized by coma and decerebrate rigidity and subsequently athony and death. | Pathological changes are usually diffuse and involve grey and white matter: Neuronal loss, gliosis, inflammation and demyelination. MV virus invades CNS and a key feature is the presence of both cytoplasmic (MV nucleocapsid) and nuclear inclusion bodies. Virus proteins are mutated and functionally defective, and no virus budding or syncytia are observed. Neurons, oligodendrocytes, lymphocytes and microglia infected by MV undergoing apoptosis. Hyper humoral immune response and intrathecal synthesis of MV antibodies. | Much of therapy in SSPE is symptom-based. To date there is not antiviral therapy of proven efficacy for SSPE. Several therapeutic agents have been used to treat SSPE, including IFN-alfa, ribavirin, Isoprinosine and levamasole. Experience has been anecdotal (due to the low frequency and variability of disease course it is hard to perform controlled clinical trials) benefit has been transient at best. | Today the only effective prevention of SSPE is to implement MV vaccination. It has been observed a marked reduction of SSPE incidence decades following MV vaccination campaigns in many countries. |

Table 1. Encephalitis Caused by Measles Virus

3. Measles Inclusion Body Encephalitis (MIBE)

The disease occurs at any age in immunocompromised patients after MV exposition. MIBE affects persons with congenital or acquired cell-mediated immunodeficiency as oncologic (approximately 70% of MIBE cases occurring in acute lymphocytic leukaemia), transplanted or HIV-infected patients. In severely immunodeficient patients the live attenuated measles vaccine in use may also cause MIBE. It has been described one fatal case of MIBE in a boy with chronic granulomatous disease after stem cell transplant without a history of recent clinical measles in the donor or the receptor (Bitnum et al.1999).

Typically, the onset occurs within one year of MV infection. MIBE may accompany or follows measles giant cell pneumonia, but more often occurs as the sole clinical manifestation, two to six months after MV infection or vaccination. Patients usually present with afebrile refractory focal seizures and altered mentation, and progress to generalized seizures, coma and death. CSF parameters are often normal and unlike SSPE hyperimmune antibody response to MV and oligoclonal bands may be not detected. EEG are abnormal, but unspecific; head computed tomography (CT) and Nuclear Magnetic Images (MRI) scans are normal. The mortality exceeds 85%, and survivors have severe neurological sequelae. At autopsy or biopsy the brain show gliosis and focal necropsia, lymphocyte perivascular cuffing, and intranuclear and intracytoplasmic inclusions in glial cells and neurons. MV nucleocapsids can be detected by electron microscopy, MV antigens by immuno-assays, and MV RNA by in situ hybridization or RT-PCR.

The virus persists and progressively invades the brain over months. At a brain autopsy from a patient with MIBE, R. Cattaneo, M. Billeter and collaborators first reported the phenomena of biased U to C RNA hypermutation in the MeV genome (Cattaneo et al, 1988). This hypermutation may take place by the enzyme Adenosine deaminase ADAR present in nervous tissue which would transform Adenosine into Inosine in the replicative intermediary RNA (see below).

4. Subacute Sclerosing Panencephalitis (SSPE)

SSPE is a rare delayed progressive encephalitis that occurs in 4 to 11 cases per 10^5 cases of measles in apparently immunocompetent children after an acute uncomplicated measles. Under 2 years of age, the risk to develop SSPE is higher, 18 per 10^5 cases of measles. The incidence has fallen drastically after successful measles vaccination campaigns. Recent epidemiological data suggest that measles vaccination protects against SSPE, and MV vaccine strain does not cause SSPE (Bellini et al, 2005; WHO, 2006; Campbell et al, 2007; Garg, 2008). The disease is caused by a persistent MeV infection that progressively invades the brain, possibly with a clonal origin, in the presence of a potent humoral response anti-MeV. The mean period from acute measles to onset of SSPE symptoms is eight years, ranging from one to over thirty years (adult onset SSPE). After a progressive course with sporadic relapses in some cases the patients usually die from few months to twenty years after the onset, although the majority of patients die between one to four years after onset of symptoms. In this chapter we describe some previous and undergoing work from our laboratory on samples obtained at autopsy from three SSPE patients (Figure 2) who presented short (3 to 4 months, SMa79), average (3.5 years, SMa84), and long (18 years, SMa94) disease course (Figure 3).

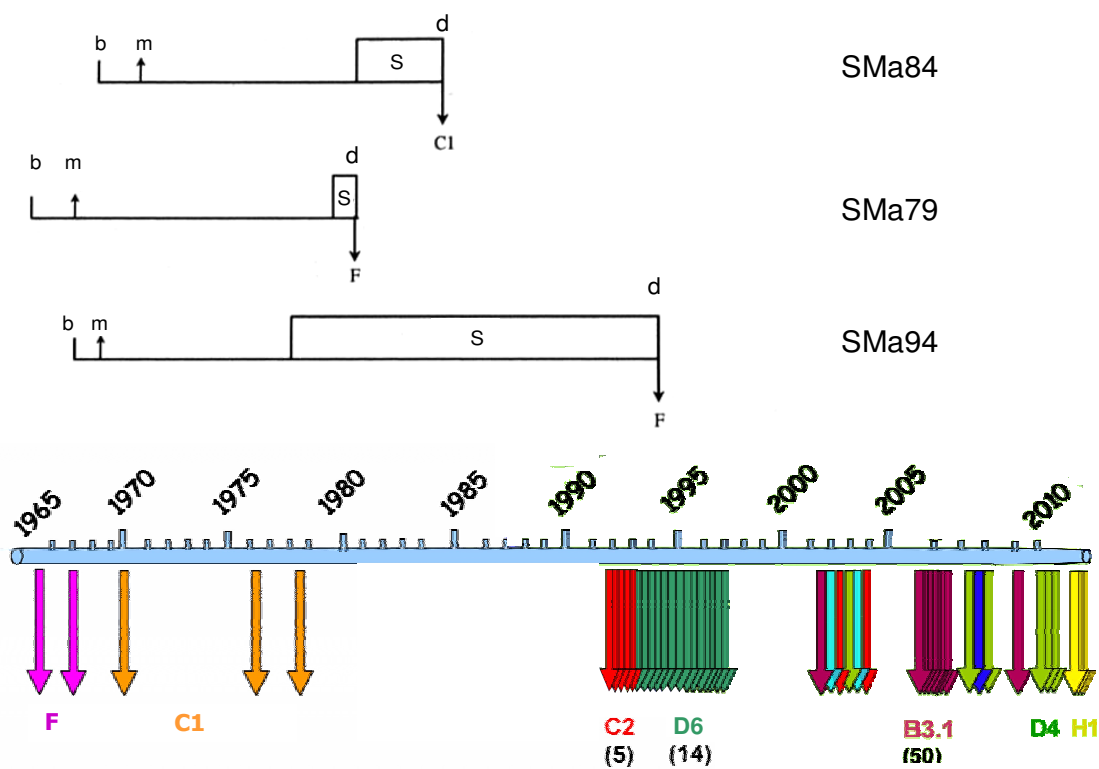


Fig. 2. Temporal distribution of measles virus genotypes circulating in a geographic area (Madrid) in the MV pre-vaccination and vaccination periods and SSPE cases. Superimposed the time-course of 3 cases of SSPE from Madrid with short duration (SMa79, 3-4 months), average duration (SMa84, 3.5 years) and long duration (SMa94, 18 years) of disease. For each patient birth date (b), date of measles acute infection (m) and dates of SSPE from onset to death are boxed (S). At autopsy, brain samples were separated and from them the whole genome sequence (15894 nucleotides for each one) was determined in our laboratory. The genotype of MV present in the brain at autopsy from each SSPE case is indicated with an arrow at the right end of the respective box. In brackets, number of isolates.

4.1 The SSPE virus

To date, MV strains present in SSPE brain tissue are wild-type strains belonging to different genotypes and no MV vaccinal strain (A genotype) has been found (Rima et al. 1995; Jin et al, 2002; Forcic et al, 2004; Mahadevan et al, 2008; Souraud et al, 2009). It is an open question the existence of circulating MV strains with high ability to establish persistent infections and cause SSPE. The epidemiological data indicate that although has been described some clusters of SSPE cases they are small and very rare, and some of them are familiar clusters (Beersma et al. 1992; Sharma et al, 2008). Reported simultaneous measles infections in identical twins with subsequent development of SSPE in only one twin do not point to the

existence of “SSPE prone” MV strains (Houff and al. 1979). These data suggest it is unlikely the existence of circulating SSPE measles virus strains prone to cause SSPE, but does not exclude they could play a co-factor role in conjunction with environmental and genetic predisposing traits in the host. In this context our group has reported a MV strain produced by a long-term persistent infection which, in contrast with the cytocidal parental virus, establishes an immediate persistence in the original human lymphoblastoid cell line (Celma & Fernandez-Muñoz, 1992). This MV strain of “persistent” phenotype establishes an immediate persistence without cytocidal effect on a number of human cell lines of different lineages (Fernandez-Muñoz & Celma, 1988). We have also observed a MV primary isolate from a clinical sample of a patient with measles that when inoculated in a human B lymphoblastoid cell line has established directly an immediate steady-state persistent infection in absence of cytopathic effects. (Fernandez-Muñoz and collaborators, unpublished results).

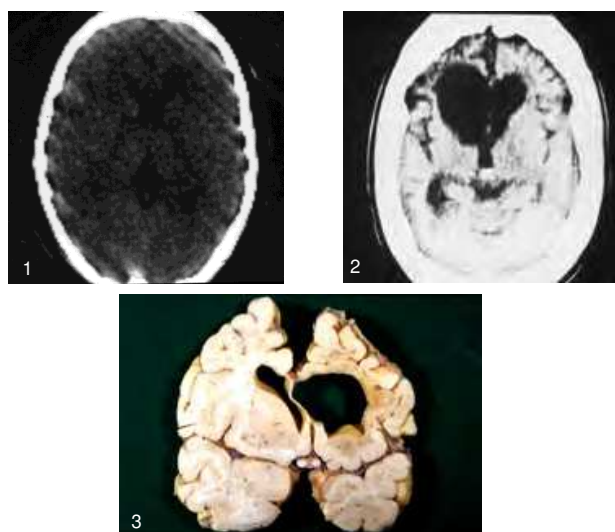


Fig. 3. Brain images of a long-course case of Subacute Sclerosing Panencephalitis (case SMa94, diagnosed and autopsied in Ramón y Cajal Hospital in Madrid). Panel 1 shows a Brain Computed Tomography (CT) image at the time the patient was 9 years old and presented a retinitis with high serum titre of anti-MV antibodies by Complement fixation and Hemagglutinin Inhibition assays, whereas serology for other neurotropic viruses was normal. One year later she presented neurologic symptoms and signs of subacute encephalitis and of intrathecal synthesis of MV antibodies was demonstrated confirming the diagnosis of SSPE. After a 20 years progressive course with transient relapses, she was hospitalized and died at Ramon y Cajal Hospital in Madrid. Panel 2 shows a brain CT image shortly before death and panel 3 shows at autopsy a sliced cerebrum from the patient. Brain atrophy with loss of white and grey matter, and ventricular dilatation were observed

(Courtesy from Dr M. García-Villanueva, Dept. Anatomía Patológica, Hospital Ramon y Cajal)

The absence of detectable virus budding and infective viral particles in the SSPE and MIBE brain and the slow course of infection indicated a defective nature of these MV strains. Studies on MV persistent infections in animal models and cultured neural and lymphoid human cells in the 1970s and 1980s, analysis of MV genomic and mRNAs present in SSPE after the development of nucleic acid amplification by PCR studies, and the rescue of infective MV from cloned genomic cDNA by M. Billeter and collaborators (Radecke et al, 1995) have provided valuable information on the pathogenesis of SSPE. Thus, amplification and sequencing of MV genomic and transcripts RNAs from diseased brain has revealed a range of mutations that could explain defective expression and functionality for several viral genes (Cattaneo et al 1988). Despite the hyperimmune response to MV antigens in SSPE patients, early observations of selective absence of antibodies to matrix protein (M) pointed to a defective expression of this protein which is necessary, among other steps, for the budding of viral particles. The search for mutation that may lead to defective budding and absence of syncytia observed in the diseased brains are concentrated in the MV viral membrane proteins: H glycoprotein, F glycoprotein, and M matrix protein.

F protein from three SSPE cases studied by Billeter et al. and two cases studied by our group (SMA79 and SMA84) presents mutational alterations in their cytoplasmic tail (Billeter et al. 1994), region which have been found involved in cell fusion (Caballero et al. 1998). In all 5 cases the SSPE F glycoprotein shows fusion activity after transfection in cultured cells (Cattaneo & Rose 1993; Carabaña, 1997).

Viral glycoprotein H from 2 of the 3 SSPE cases from Madrid (SMA79 and SMA94) showed mutations in their distal cytoplasmic region with extension of 4 amino acids due to mutation in the stop translation codon (Carabaña, 1997, and Celma et al unpublished results).

In a MV matrix gene from brain of a child with MIBE, M. Billeter and collaborators described for the first time the phenomenon of biased hypermutation, a cluster of exceptional point mutations (50% of U residues were changed to C); these biased mutations found only in the M gene could confer to the MV an selective advantage in the brain (Cattaneo et al. 1988). Wong and collaborators (Wong et al. 1989) shortly afterwards, found a non-random M gene hypermutation, similar to the one identified by Billeter, in a SSPE virus strain (Yamagata-1) passaged in human neuroblastoma cells.

Biased hypermutation was reported for the first time in the brain of a SSPE case by Celma and collaborators in the matrix protein M gene of SMA84 (Carabaña et al., IX International Congress of Virology, Glasgow, 1993). 38% of the U residues mutated to C, generating 59 amino acids changes in M protein, 18 of the changes being Leu to Pro, which probably altered drastically its secondary structure. Figure 4 shows a map of the different type point mutations found across the entire genome sequence in 3 SSPE cases. In SMA94 we observed biased hypermutation U to C at a lower level, 10% U to C, producing 12 aminoacid changes; besides the hypermutation, there is a loss of the 83 amino acids at the C-end. In contrast, in SMA79, there is not biased hypermutation, and we found the creation of a premature stop triplet that caused the loss of 40 residues at the C-end of M protein. In this SSPE case, M protein presented a drastically impaired interaction with MV nucleocapsids tested by binding of radio-labelled cloned M proteins to purified MV nucleoprotein (Carabaña, 1997, and unpublished results). Thus in SSPE, 1. biased hypermutation was not dependent of MV genotype, 2. bias hipermutation may requires several years course of disease, and 3. the level of final hypermutation do not increase necessarily with the length of the disease (Figure 4).

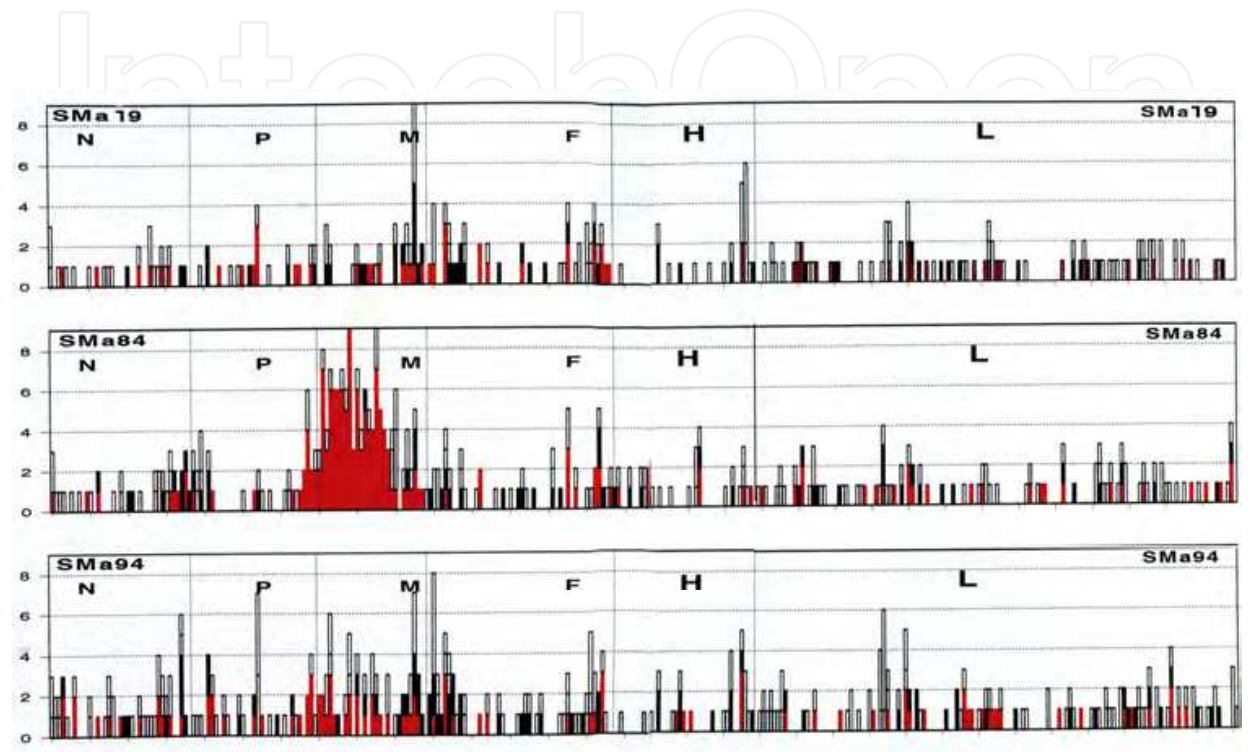


Fig. 4. Distribution of mutation events in MV Genome of SSPE cases with short, average and long disease course. The antigenome of SMA79, SMA84 and SMA94 are represented. Point mutations U to C (red boxes), C to U (black boxes), G to A (grey boxes) and others (white) are indicated. The height of boxes correspond to the number of mutated bases. On the horizontal axis, groups of 50 consecutive nucleotides were taken as a unit. Gene junctions are indicated by vertical lines. The methodology used for determination of intergenomic regions and whole genes were as described for analyzing MV genotypes (Rima et al.1995). RT-PCR products were sequenced directly in both directions by dideoxynucleotide chain-terminating sequencing with primers constructed at 400-500 base intervals and manual analysis or by using an automated DNA sequencer (model ABI PRISM 377 Applied Biosystems). Sequences of 5' and 3' end of the SSPE genomes were determined by a ligation method (Sidhu et al. 1993) and a modification of the 5'RACE method (Frohman et al. 1988, Baron et al. 1995, Carabaña,1997).

The same hypermutation pattern was observed in distant zones of brain for each case, in agreement with previous results from Billeter, ter Meulen and collaborators (Bazko et al. 1993), suggesting a clonal origin of brain infection in SSPE. Biased hypermutation was not observed outside the M gene in any of the studied cases. (Carabaña, 1997, and Celma and cols, unpublished results). It remains unknown whether the M gene transcription is particularly susceptible to hypermutation or the process is frequent for every MV gene, but hypermutation in other viral genes generates unfit genomes which are counter-selected in the brain environment. A possible mechanism for this biased hypermutation was proposed by M. Billeter group in collaboration with H. Weintraub group (Bass et al, 1988). Essentially, the mechanism consists in the action of Adenosine-deaminase that converts in double stranded RNA Adenosine into Inosine resulting in a transition A to G or U to C. This enzyme found by Weintraub group has been later identified as ADAR1, a member of ADAR protein family inducible by Interferon I. While ADAR1 activity in cell culture has been reported to be barely detectable (Horikami & Moyer, 1995), recent studies in transgenic mice indicate that ADAR1 is a restriction factor controlling the replication of MV and other Paramyxovirus. On the other hand there are indications that ADAR1 is a proviral antiapoptotic host factor in the context of MV infection (Ward et al, 2011; Toth et al. 2009; and Samuel, 2011 for a recent review).

Since 1954 when von Magnus first described in influenza virus subgenomic nucleic acid particles, defective interfering particles (DI) have been found in a variety of viruses. In 1970 Alice Huang and David Baltimore suggested that DI particles play a role in the establishment of viral persistent infections. E. Norrby described MV DI particles, and in 1977 Rima, Martin and collaborators demonstrate a role of DIs in establishment of MV persistence in cultured cells (Rima et al, 1977). DI particles were detected in measles vaccine pharmaceutical preparations by Roux and coll. (Calain & Roux, 1988). In 1992 Fernandez-Muñoz and Celma did not find evidence of the subgenomic RNAs at detectable level in MV metabolically radio-labelled nucleocapsids by denaturing formaldehyde gels from a long-term steady-state MV persistent infection in human lymphoblastoid cells with reduced level of infective virus (Fernandez-Muñoz & Celma, 1992). In 1994 Dowling, Udem and collaborators with dot-blot hybridization and RT-PCR experiments found overrepresentation (2 to 5 times) of MV genomic 5' end sequences and the presence of copy-back type DI particles in MV nucleocapsids from brain material of 3 SSPE cases (Sidhu et al, 1994).

In post mortem obtained brain samples from 3 SSPE cases of short (SMA79), average (SMA84) and long (SMA94) course of disease (Figure 2), we have searched for MV defective particles. Genomic RNA (-) from purified nucleocapsids was hybridized with radio-labelled (32P) RNA probes with polarity (+) designed to recognize different MV genes along the viral genome to determine the abundance in the brain of the regions of MV genome. As shown in Figure 5 no detectable overrepresentation of 5' or 3' ends of MV genome was observed in any of the 3 SSPE cases, indicating that no abundant DI particles of deletion type were present. To assay specifically for the presence of copy-back type MV DI particles we amplified by RT-PCR MV nucleocapsid RNA with suitable primers of the same polarity and products were detected by liquid hybridization with radio-labelled probes. Only in one SSPE case, SMA84 with clinical course of average length, were detected copy-back DI particles (Figure 6). Sequencing of amplified cDNA corroborated the copy-back structure of the subgenomic RNA and determined the polymerase jump point. Thus, the presence of

copy-back DI particles in the brain is not universal in SSPE cases, and its presence is not associated with long clinical course of disease. The possible functional interfering ability of these copy-back DI particles that could modulate MV replication in SSPE remains conjectural.

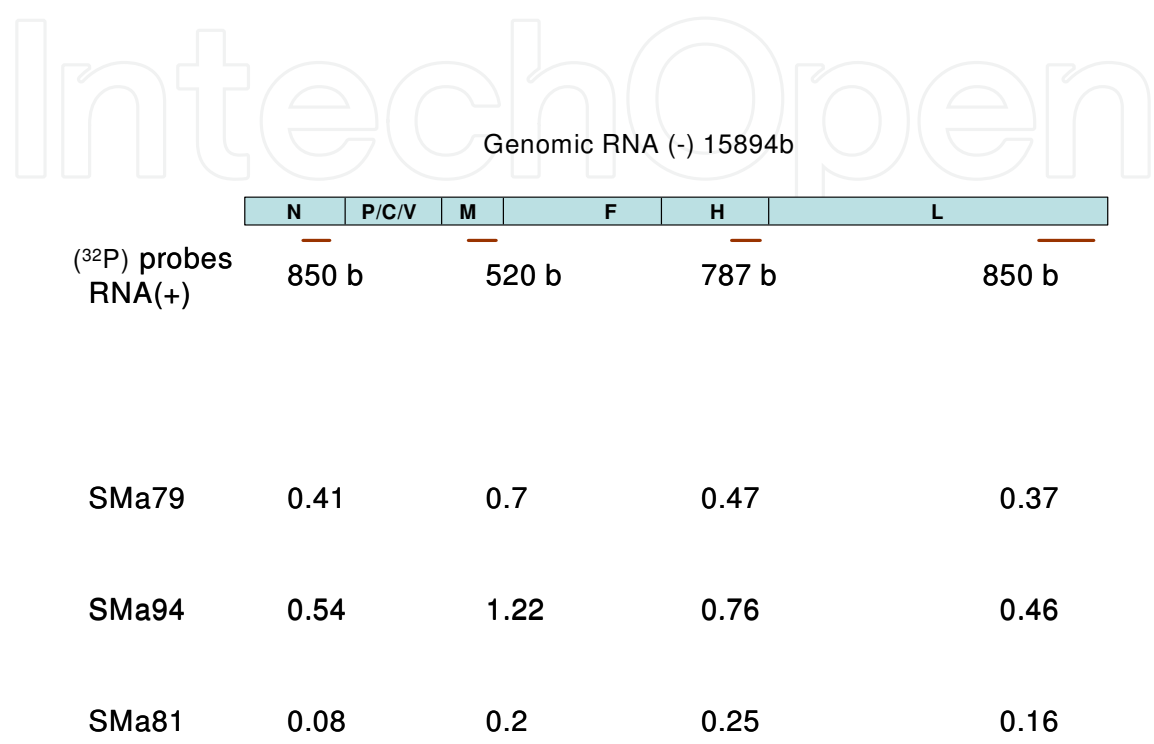


Fig. 5. Search for possible subgenomic MV in SSPE brains. Hybridization with labelled probes across MV genome show that different regions are similarly represented in genomic RNA (-) from brain material of SSPE cases with short (SMa79), long (SMa94), and average (SMa84) disease course. Positions of the RNA probes for four MV genes (top) and the fentomoles of each gene hybridized per 10µg of Brain Nucleocapsid RNA (bottom). Genomic RNA and labelled RNA standards of antisense polarity were slot blot hybridized with P³² sense polarity probes and radioactivity estimated by densitometry analysis (Carabaña 1997). Genomic RNA from postmortem SSPE brain tissue was purified from nucleocapsids isolated by CsCl gradients. Probes and standards RNA specific for nucleoprotein (N), Matrix (M), Hemagglutinin (H), and polymerase (L) was of obtained by transcription with T7 and SP6 polymerases of Gemini vectors (Celma and Fernandez-Muñoz 1992) containing from nucleotide 825 to 1676 for N gene, from 4215 to 4719 for M gene, from 7669 to 8456 for H gene (generous gift from Dr.M.A.Billeter) and a clone obtained in our laboratory containing a 3´terminal fragment of gene L from nucleotide 14892 to 15742.

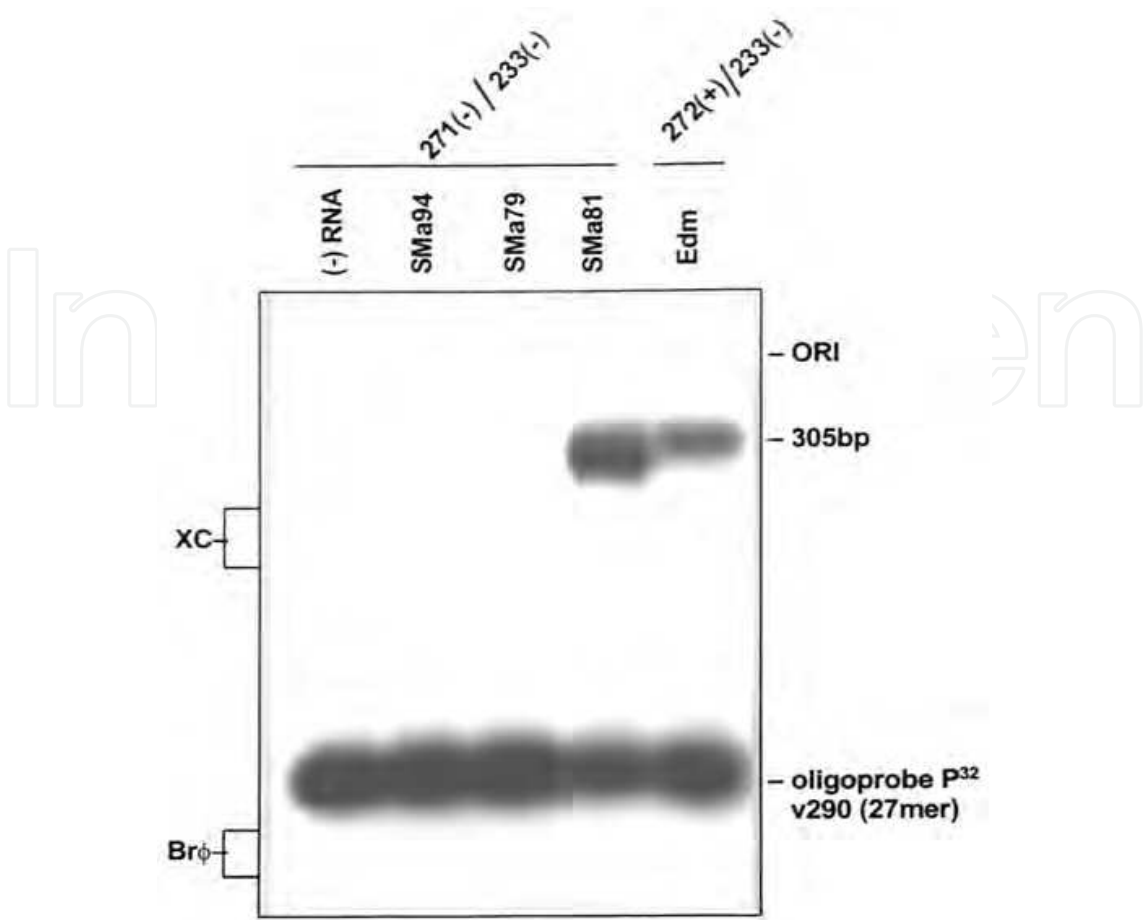


Fig. 6. Copy-back defective measles virus particles in SSPE brain. Liquid Hybridization autoradiograph of 5' copy-back genome specific PCR products synthesized with primers designed to amplified copy-back DIs. The determination in our laboratory of the entire sequence of MV genomes from brain of 3 SSPE cases allowed us to synthesize transcription vectors from which riboprobes specific for their genomic 5'end region were synthesized (Carabaña J. 1997). Defective viral products were amplified by RT-PCR of Nucleocapside RNA with primers of same polarity as described by Calain et al.1992 and Sidhu et al.1994. Products amplified with primers V233 (antigenome nucleotide numbers15894 to 15866) and V271 (15648 to 15621) were detected by liquid hybridization with internal probe V290(15783 to 15812). Positive control hybridization from Edmonston measles virus RNA amplified with primers of both polarity V272 (15588 to 15613) and V233 is shown. Based on the sequence of amplified DI copy back, the polymerase jump point position is 15764 and15570. Assuming the DIs follow a precise copy-back mechanism and their generation assumes complete complementarity of the ends, the size of the detected Sma84 DI particles were 456 nucleotides. Detection of copy-back DI particles was observed in only one of the three SSPE cases studied.

4.2 The SSPE patient

The SSPE patients were otherwise healthy individuals who has a history of past acute measles, typically uncomplicated and before the age of two; SSPE affects males preferentially, ratio 2.5:1. Several hypothesis have been proposed to explain the epidemiological data of early MV infection as a predisposing factor for SSPE development;

for instance it has been suggested, an still immature immune response, anatomical factors that favor viral invasion of CNS, or the presence of maternal anti-measles antibodies which could modulate the MV infection. So far these hypotesis remains unproven. Another conceivable possibility would be the coincidence of the acute MV infection with another infection by a different pathogen which could facilitate the MV persistence and invasion of the CNS. Were this the case, as coincident infections may be more frequent at an early age, the coinfection hypothesis could explain the association of SSPE development with an acute measles early in life. In our laboratory we have been testing during the last three decades this hypothesis in a collection of samples from SSPE patients diagnosed along those years, but so far we found no conclusive results. In 2005, based on the association between the development of SSPE and the immunodepression by intensive immunosuppressive therapy in unimmunized subjects, or on the early age immaturity of immune system, M. Oldstone and collaboratos proposed SSPE likely arises after MV infects an transiently immunosuppressed individual; these authors developed an SSPE transgenic mouse model expressing CD46 MV-receptor transiently immunodepressed by prior infection with lymphocytic coriomeningitis virus (LCM -Cl 13), proposing a dual viral hit playing a role in causation of SSPE (Oldstone et al, 2005; Oldstone, 2009). This model promises to be useful for pathogenesis studies and assays in search of effective new therapeutic approaches to SSPE and MIBE.

It is conceivable that some individuals have genetic traits that predispose them to develop SSPE after MV exposure. In recent years several polymorphisms have been associated with SSPE from different populations. Thus, several functional polymorphisms in the regulatory regions of genes for the expression of proteins involved in the immune response as MxA (protein associated to the anti-viral response induced by Interferon I), Interferon Regulatory Factor 1 (IRF-1), Interlekin-4 (IL-4), Toll-like receptor 3 and granulysin are associated with development of SSPE in the Japanese and Filipino populations. On the other hand polymorphisms in IL-12, IL-2, Interferon-gamma, Angiotensin-converting enzyme (ACE) and Angiotensin II type 1 receptor have been associated with SSPE in Turkish population (Kusuhara et al, 2007). More recently these authors have obtained results in Japanese and Filipino populations suggesting that PD1 gene may contribute to genetic susceptibility to SSPE. Due to the small size of some of these samples, further studies are needed to confirm these associations and their significance for the development of SSPE.

4.3 SSPE onset, clinical course and pathogenesis

Onset occurs on the average 8 years after MV acute infection, ranging from one year to decades in adult-onset SSPE; in rare cases onset occurs during pregnancy and it is often fulminant. The course of SSPE is progressive for one to twenty years, sometimes with transient remission periods, but in most cases death occur within 3 to 4 years of onset. The course of SSPE use to be divided in 4 stages with rare transient remissions periods (Garg, 2008). Stage 1. The onset is insidious with symptoms of progressive cortical dysfunction, behavioural changes, deterioration of intellectual capacity, and some times awkwardness, stumbling or visual symptoms of retinitis, optical neuritis or cortical blindness, over months. Stage2, Later, manifest motor disability and paroxysmal disorder develop: mioclonus jerks (pathognomonic electroencephalographic alterations-Rodermacker complexes). Stage 3. Pyramidal and extrapyramidal manifestations, disappearance of myoclonus, alteration in sensorium. Stage 4. Vegetative state, and death.

The diagnosis of SSPE can be established with the compliance of the following diagnostic Dyken's criteria: 1. Atypical clinical picture of progressive subacute mental deterioration with stereotyped generalized myoclonus. 2. Characteristic electroencephalogram changes. 3. Elevated CSF globulin levels greater than 20% of total protein. 4. Raised CSF anti-MV antibody titers (intrathecal synthesis of measles antibodies) 5. Typical histopathologic findings in brain biopsy or autopsy; pathological changes are usually diffuse and involve grey and white matter: neural loss (Figure 3), gliosis, inflammation, and demyelination. A key feature is the presence of both cytoplasmic and nuclear inclusion bodies, predominantly in neurons and oligodendrocytes. No budding of MV particles or syncytia cytoplasmic effects are observed, and scarce neurons, oligodendrocytes, microglia, and lymphocytes infected by MV undergo apoptosis.

When and how MV enter into the CNS in SSPE remain unknown questions, but molecular epidemiology and *in situ* studies have provided some likely answers. A basic question is whether the MV causing SSPE is the same virus that caused the acute measles in the patient years before. This is a difficult question to answer, especially in SSPE presenting a long course, since is improbable to have for one patient both the primary MV isolate causing the acute infection and the virus recovered from his brain years to decades later. The recognition of MV genotypes, and their geographical and temporal distribution pattern (rapid "endemic" genotype replacement after years of circulation) in the pre-vaccination period provided an answer to this question (Rima et al, 1995). Thus, studying the MV genotypes that circulated in a large city like Madrid from 1960s to 1990s and 3 local SSPE cases from this period (Figure 2), we observed that the genotype of the MV recovered at autopsy from the brain of each SSPE patient was the same genotype circulating in Madrid at the documented date of his acute infection at early infancy, and not the one circulating in Madrid at the date of onset of SSPE years later. This was the first confirmation that a SSPE is long-term infection by MV, and that it is not caused by a MV re-infection, representing the prime example for a long-term persistent human infection by an RNA virus, (Rima et al, 1995; Carabaña, 1997).

The question arises, where MV resides and replicates in an individual during the intervening years between acute infection and onset of SSPE symptoms. One possibility would be that during acute measles the virus enter into the CNS of the subjects who will develop SSPE. This hypothesis is based on data from post-mortem brain samples from SSPE cases where cerebral vascular endothelial cells showed infection by MV (Kirk et al.1991), and in acute fatal infection cases where MV infected cerebral endothelial cells were found by *in situ* hybridization and *in situ* RT-PCR (Esolen et al, 1995). This site of infection may provide a portal of entry for MV in subjects who subsequently would develop SSPE or MIBE or a target for immunological reaction in ADEM. Although these epithelial cells do not express the CD150(SLAM) receptor, they could be infected through recently discovered CD147/EMMPRIN receptor expressed in epithelial cells (Watanabe et al, 2010).

MV is highly lymphotropic (Moench et al, 1989) and the virus infect monocytes, lymphocytes and possibly dendritic cells early in the natural acute infection and in experimental animal infections. The data after aerosol infection of non-human primates strongly suggest that MV entered the host at the alveolus by infecting macrophages or dendritic cells which traffic the virus to local lymph nodes, resulting in a primary local amplification and subsequent systemic dissemination by cell-associated viremia (Ferreira et al, 2010; Lemon et al, 2011). In patients with measles the clearance of detectable RNA by RT-

PCR in MV-infected blood cells may occur after several months of acute infection (Ridell et al, 2007). From these results, it is conceivable that MV infecting mononuclear cells as monocytes could survive as long-lived macrophages for months or years invading by a trojan horse mechanism different organs, including the brain in some patients. In our laboratory we have established long-term steady-state persistent infection in a number of human monocytic cell lines (Ortego, 1994); in some of them we observed cell-surface over-expression of cell adhesion molecules as Intercellular Adhesion Molecule 1 (ICAM-1) or integrin LFA-1, which could facilitate the attachment of infected leukocytes to endothelial cells (Fernandez-Muñoz et al. unpublished results). On the other hand, we differentiated in vitro the MV-persistently infected human monocytic cell lines to macrophage-like cells by means of PMA or GM-CSF which kept expressing high levels of MV proteins for weeks in the follow-up (Ortego, 1994, and Fernandez-Muñoz et al. unpublished results). These results suggest that MV infected monocytes may be converted to macrophages which could remain infected by MV and might harbour the virus for years. Previous results suggesting that MV was present in peripheral blood mononuclear cells (PBMC) and lymphoid organs from some SSPE patients (Brown et al, 1989), have not been confirmed (Schneider-Schaulies et al, 1991, Rima & Duprex 2005 for a review). In an early study by J. Sever and collaborators MV was isolated in mixed cultures of HeLa cells with lymph node biopsies from 2 out of 5 SSPE patients (Horta-Barbosa et al, 1971). As the isolated MV hemagglutinated macacus rhesus erythrocytes, it is possible that the isolates were a MV vaccine strain contamination (Lecouturier et al 1996). As we had the opportunity to be present at the time autopsy was performed for patients SMa84 and SMa94, we could collect “clean” extra cranial tissues before the braincase was opened to obtain brain samples. Thus, among other samples, we collected separately thoracic and mesenteric lymph nodes from these SSPE patients. From every lymph node we amplified by RT-PCR MV genomic (-) RNA from N, P, M, F and H genes (Figure 7). The MV in lymph nodes belongs to the same genotype that the MV in the respective brain, C1 in SMa84, and F in SMa94, and had high sequence homology with the respective MV in the respective brain. In both patients we have detected differential mutations between lymph nodes and brain in genes N, P and M, some of them resulting in aminoacid change. Interestingly, in both patients MV found in lymph nodes showed biased hypermutation U to C in the M gene, and in the SMa94 lymph nodes M gene, besides having all mutation found in brain, there are 10 additional mutations, all of them T to C mutations, and 7 resulting in change of aminoacid (Carabaña 1977, Celma et al. unpublished results). Although the separate collection of extra brain tissues and the diversity of sequences obtained, did not indicated possible contamination with brain RNA, to further exclude it we designed and performed in parallel amplification from lymph nodes RNA of Glial fibrillar acidic protein, abundant protein in nervous tissue, that resulted lower than amplification from PBMC from a control sample (Fig. 7). Although our results indicate that MV is present in lymph nodes of SSPE patients at the late stages of the disease, so far can not answer the question where the virus resides and replicate in the patient the years elapsing between the acute infection and the onset of encephalitis. The comparative analysis of genome sequence of two coetaneous MV belonging to the same genotype, F, one causing a short course (months) SSPE (SMa79) and the other one causing a very long course (twenty years) SSPE (SMa94) might shed some light on this issue (Celma et al, work in course). The study of MV sequences present in different zones of brain from one SSPE patient suggest that the invasion of CNS by MV has a clonal origin (Bazcko et al. 1993; Carabaña, 1997).

MV dissemination through brain in SSPE. The presence of MV nucleocapsids and MV Hemagglutinin in the neuronal axonal processes suggest that MV spreads transneuronally (Rima & Duprex, 2005 for a review). In 1990 D.Payan and collaborators found that a lymphoblastoid cell line that constitutively express the neuropeptide, (substance P) receptor, neurokinin-1, facilitate MV fusion (Harrowe et al, 1990). M. Billeter and cols in slice cultures demonstrated neuron-to-neuron polarized spread of a recombinant autofluorescent MV (Ehrengruber et al, 2002). More recently, G.Rall showed in a transgenic mice model implication of neurokinin-1 in infection and spread of MV, serving as viral receptor, or co-receptor in neurons, allowing MV synapsis (Makhortova N et al, 2007). No doubt the integration of these approaches will shed light on the pathogenesis of SSPE and other viral encephalitis.

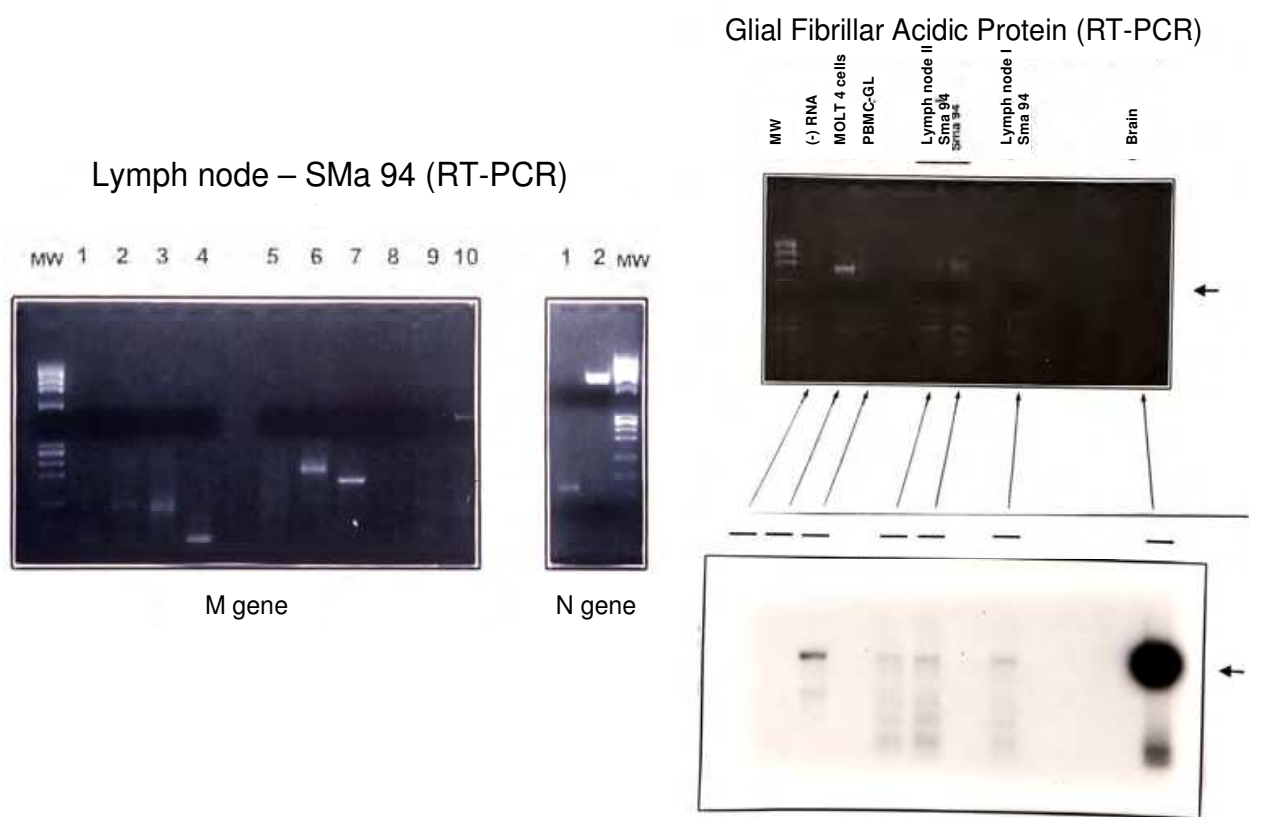


Fig. 7. Detection of genomic MV RNA in lymph nodes of SSPE patients. Agarose gel electrophoresis of cDNA amplified for MV M and N genes of a lymph node from SMa 94(left). Analysis of fibrillary acidic protein gene in SMa94 lymph nodes I and II by amplification and hybridization with specific primers (right).

Left panel. At autopsy mesenteric lymph nodes were obtained before opening the braincase. RNA was extracted using guanidinium isothiocyanate technique, reverse transcribed, amplified (1,2,3,4) and re-amplified (5,6,7,8,9,10) with specific genes for different regions of gene M and N (1,2). Negative controls 1M, 5M and 1N and MW markers. Sequencing the amplified DNA for N gene, besides the nucleotide changes characteristic of brain genotype there is one aminoacid change Ser432 to Leu. For M gene, from 1 to 135 residue, there are 8 aminoacid changes with respect to the brain sequence.

Right panel. To further exclude any MV RNA from brain samples glial fibrillary acidic protein mRNA was amplified. This transcripts are abundantly represented in brain RNA and scarcely in lymphoid tissue; agarose gel electrophoresis of RT-PCR amplification with fibrillary acidic protein gene specific primers (Reves et al. 1989) (top) of RNA from MOLT4 as negative control, RNA from a control sample of peripheral blood mononuclear cells (PBMC), RNA from two lymph nodes (I,II) and SMA 94brain RNA. Analysis of the amplified material by hybridization with specific radiolabelled probe (bottom). Carabaña1997.

5. Antiviral therapeutic approaches to encephalitis caused by measles virus infection of the central nervous system: SSPE and MIBE

Multiple therapeutic agents, including Interferons, Ribavirine, Isoprinosine, vitamin A have been used to treat measles complications, including SSPE and MIBE, but benefit has been transient at best. Today there is not antiviral therapy of proven efficacy for MV. We will briefly review past experience, some times necessarily anecdotal, given the low frequency and highly variable course of these diseases that hinder controlled clinical trials. We will discuss new potential anti-MV therapies including, RNA interference, inhibitors of virus entry and MV RNA polymerase, (reviews by Garg, 2008; Pempfer & Snyder, 2009; Reuter & Schneider-Schaulies, 2010) and a novel therapeutic approaches including selective induction of apoptosis in MV infected cells as a potential early treatment of SSPE and MIBE.

5.1 Small molecules and natural products with anti measles activity

Ribavirin. This pro-drug analogue of ribonucleosides with a broad antiviral spectrum has been used alone or combined with Interferon-alfa by intra-ventricular administration for SSPE patients with variable results, transient benefit at best, and undesired effects. Experimental results in MV intra-cranial infected hamsters and mice have shown that complexation of ribavirin with cyclodextrin-alfa reduced five-fold the 50% inhibitory dose and improved crossing of the brain-blood-barrier (Jeulin et al., 2009), and could improve the treatment with ribavirin in MV encephalitis.

Vitamin A. Supplements of Vitamin A significantly reduce measles mortality and morbidity, especially in children younger than 2 years of age, and it is the treatment recommended by WHO for children suffering from acute measles (Joint WHO-UNICEF statement-1987-Vitamin A for measles. Wkly Epidemiol Rec 19,133-134). There are indications of Vitamin A playing a role in the innate immune response, particularly in Interferon I signalling pathway, and it has been reported that retinoids directly inhibit MV replication in cultured cells (Trottier et al, 2009). On the other hand, it has been reported about one third of a SSPE (6 of 21) and (0 of 20 matched controls) showed low levels (<20micrograms per dL) of vitamin A (Gugor et al, 2007). It remains an open question whether Vitamin A supplements might implement the Intereferon treatment in some SSPE patients.

Inhibitors of MV entry or MV RNA-polymerase. During the last decade a number of small molecules strong inhibitors of MV entry or viral RNA-dependent RNA polymerase as AS-136A have being designed by R. Compans, R. Plemper & collaborators. Targets in MV RNA-polymerase L protein catalytic subunit were identified studying in cell cultures MV escape mutants to the antiviral. The emergence of MV escape mutants could be a draw-back in

potential long treatments as for SSPE. It remains to test these drugs in MV persistent infections in animal models for SSPE (for a review, Plemper & Snyder, 2009).

5.2 Interferon- α

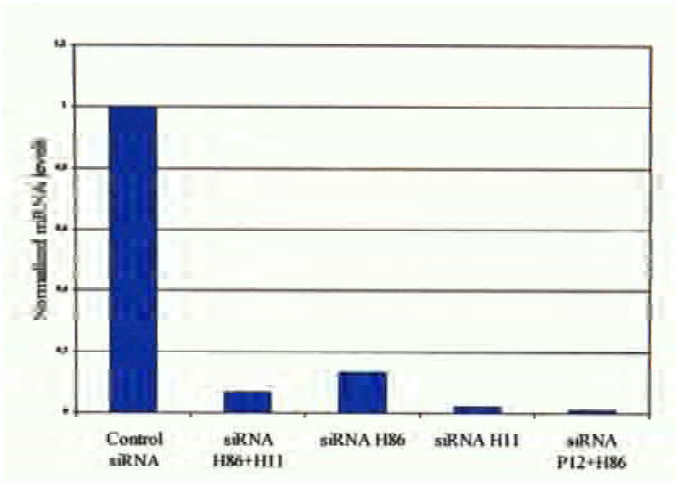
In SSPE patients treatments with intraventricular Interferon- α alone or in combination with ribavirine or isoprinosine produce at best transient effects, and in some cases severe toxic effects (for review, Garg, 2008; Nacagawa et al 2009). Possibly, the low antiviral effect of Interferon- α treatment could be explained by the MV inhibition of antiviral response to exogenous Interferon I in the infected cell (Ortego, 1994 Fernandez-Muñoz et al, 2000). We found that MV inhibited the antiviral response to IFN I by blocking the signal transduction from the IFN I-receptor (Liton, 2001), and that MV non-structural protein V is associated with this inhibition (Celma and collaborators, unpublished results; Palosaari et al. 2003)). Silencing the V protein expression by anti-sense RNA oligonucleotides or RNA interference may be a way to increase IFN I antiviral effect in SSPE treatment. With this aim we have designed siRNAs that block MV P gene expression that could render MV persistently infected cells sensitive to IFN- α (see below). Another possible factor for the low antiviral response to Interferon I in SSPE patients could be the functional MxA promoter polymorphisms associated with SSPE (Torisu et al, 2004).

5.3 RNA interference to control progression of SSPE and MIBE

The gene suppression effects mediated transiently by short interfering RNA molecules (siRNA) or stably by intracellular expression of short hairpin RNAs (shRNAs) which are processed by the cellular RNAi machinery (for a review Dykxhoorn et al. 2008) into effective siRNAs, are currently being tested as therapy for acute virus infections such as RSV and for chronic infections as HIV, Hepatitis B virus and Hepatitis C virus. To determine whether exogenous siRNA could inhibit the expression of MV genes and suppress viral replication during acute and persistent infections, we have designed siRNA molecules targeting conserved sequences in the genome of MV in brain of SSPE patients which inhibit the expression of MV Phosphoprotein gene, involved in viral RNA transcription, replication, and IFN response, and Hemagglutinin gene(H), playing a critical role in adsorption, cell fusion, assembly and budding of viral particles (Martín-Cortes et al, 2004 and Celma and coll. unpublished results). As shown in Figure 8 these siRNAs efficiently inhibit the production of MV infective particles in acute and persistently infected cells and indicates could be an useful tool for antiviral therapies by themselves or in combination with others MV specific siRNAs (Reuter et al. 2006; Otaki et al. 2007; Keita et al. 2008). For an efficient siRNA therapy besides a high gene target specificity it will be necessary to solve problems of siRNA delivery and undesirable toxic site effects, as discussed by Rossi et al 2009.

5.4 Inducers of apoptosis in MV infected cells as a potential early treatment of SSPE and MIBE

Formation of syncytia by inducing cell fusion is the prominent cytopathic effect of MV in cultured cells (Enders, 1954) and in patients with measles giant cell pneumonia. In our laboratory by infecting a series of human lymphoblastoid cell lines with a MV strain showing low cell fusion activity, we observed that infected MOLT3 cell line underwent an atypical rapid cytopathic effect without a significant formation of syncytia that we described at the 1988 Negative Strand Viruses Conference (Fernandez-Muñoz et al. 1988).



Titer of extracellular virus (%inhibition)

| | Control siRNA | siRNA-P12 | siRNA-H86 |
|---------------------------------|---------------|-------------|------------|
| Lytic infection (293+EdMeV) | 1:192 | 1:24 (87%) | 1:48 (75%) |
| Persistent infection (293-FV-P) | 1:1024 | 1:128 (87%) | ND |

Table 1: Effect of siRNA-P12 and siRNA-H86 on production of infective extracellular MeV in lytic and persistently infected 293 cells.

Fig. 8. Inhibition by small interfering RNAs (siRNAs) of MV gene expression and viral replication during acute and persistent infections. Based on conserved sequences among MV primary isolates from patients with acute measles or SSPE we have designed siRNAs ds-oligonucleotides complementary to MV Phosphoprotein (siRNA P12) and Hemagglutinin (siRNA H86 and H11). Human epithelial 293 cells lytically infected with Edmonston virus or persistently infected with MV isolates were transfected with chemically synthesized siRNAs using Lipofectamin 2000. Upper panel. Quantitative assay to measure hemagglutinin gene silencing by siRNA. MV-H mRNA was assayed by quantitative reverse-transcriptase-polymerase-chain-reaction using SYBR Green core reagents from Applied Biosystems after primer optimization and Actine or GAPDH as endogenous controls in a ABI Prism7000 Sequence Detection System. Each column shows the relative quantification for Hemagglutinin mRNA following transfection of 293-FV-P persistently infected infected cells with the indicated siRNA.Lower panel. Effects of siRNA-P12 and siRNA H86 on production of infective extracellular MV during acute and persistent infections. Cell supernatants were titrated by plaque assay on B95 cells adapted in our laboratory to grow in monolayers.

Attempting to characterize this previously un-recognized MV cytopathic effect we found in MOLT3 infected cells chromatin condensation and DNA inter-nucleosoma fragmentation, hallmarks of the cell death mechanism described and named apoptosis by J.Kerr and collaborators in 1972. Measles virus can induce apoptotic cell death in cultured human cells and this process is mediated by over-expression of Fas membrane protein in MV infected lymphoid cells (Fernandez-Muñoz et al., Ninth International Conference on Negative Strand Viruses, Estoril, 1994). At this conference apoptosis induced by MV was also present by Dr D. Griffin in Vero cultured cells (Esolen et al, 1995; Caballero et al, 1996). Based in our previous observation of Fas (CD95) involvement in apoptosis caused by MV, we studied the effect of Fas ligand (FasL) and other analogs as TRAIL (TNF-related apoptosis-inducing ligand) on acute and persistently infected human cells. We observed that MV persistently infected cells were more sensitive to apoptosis induced by exogenous TRAIL than uninfected cells (Figure 9). This sensitization could be explained by the up-regulation of functional TRAIL receptors TRAIL-R1 and TRAIL-R2, and down-regulation of anti-apoptotic factor bcl-2 and activation of protein-kinase Akt and NFkB (Duque et al, 2007, and unpublished results by Celma and collaborators). Since has been generally observed that cancer cells are more sensitive than normal cells to apoptosis induced by recombinant TRAIL this molecule has been object of numerous clinical trials. Although phase I trials have shown low TRAIL toxicity, the efficiency tests got mixed results, largely due to the development of tumours resistance to the action of TRAIL (Yagita et al 2004 for a review, Kim et al. 2008; Eaton et al, 2011). Given the lack of efficient therapies for the encephalitis caused by persistent infection for MV, MIBE and SSPE, we have proposed the potential use of TRAIL as an early treatment of these diseases with the object to kill selectively the cells where MV resides before the virus disseminates across the brain.

TRAIL and its receptors have been shown to play important roles in the immune response to viral infections and in immune surveillance of tumours and metastasis (Falschlehener et al, 2009). During the last decade several studies have shown that different viral infections sensitize cells to apoptosis induced by TRAIL. Thus, TRAIL-resistant fibroblasts could be sensitized to TRAIL-induced apoptosis by infection with human cytomegalovirus (Sedger et al. 1999). On the other hand, it was observed and strong up-regulation of TRAIL, TRAIL-R1, and TRAIL-R2 in response to respiratory syncytial virus in primary tracheal-bronchial cells, A549 and HEP-2 cells and, RSV-infected cells could be eliminated by TRAIL-expressing immune cells in vivo (Kotelkin et al 2003). Furthermore, TRAIL has been implicated in chronic HCV infection and HCV has been shown to sensitize human hepatocytes to TRAIL induced apoptosis (Lan et al, 2008). Thus, the approach of an early treatment with TRAIL could help to control persistent infections by different viruses. However, there are some motives for concern after recent results showing that TRAIL, in addition to anti-tumour activity, has immunomodulatory functions and it has been demonstrated that TRAIL can eliminate plasma cells in vitro and suppress antibody production in vivo. Therefore, it should be noted that a strategy to over-express endogenous TRAIL, as well as administration of rTRAIL may impair host defense against infection (Faschlehener et al, 2009). For treatments of SNC diseases, some findings in cultured brain slices raise concern about neuro-toxicity and argue against the use of TRAIL for therapy of human brain tumours (Nitsch et al, 2000). A recent study obtained successful results combining anti-papillomavirus E6/E7 siRNA and TRAIL induction of apoptosis in cancer cells being refractory to TRAIL treatment (Eaton et al 2011).

MeV sensitizes Human Epithelioid 293 to TRAIL induced apoptosis

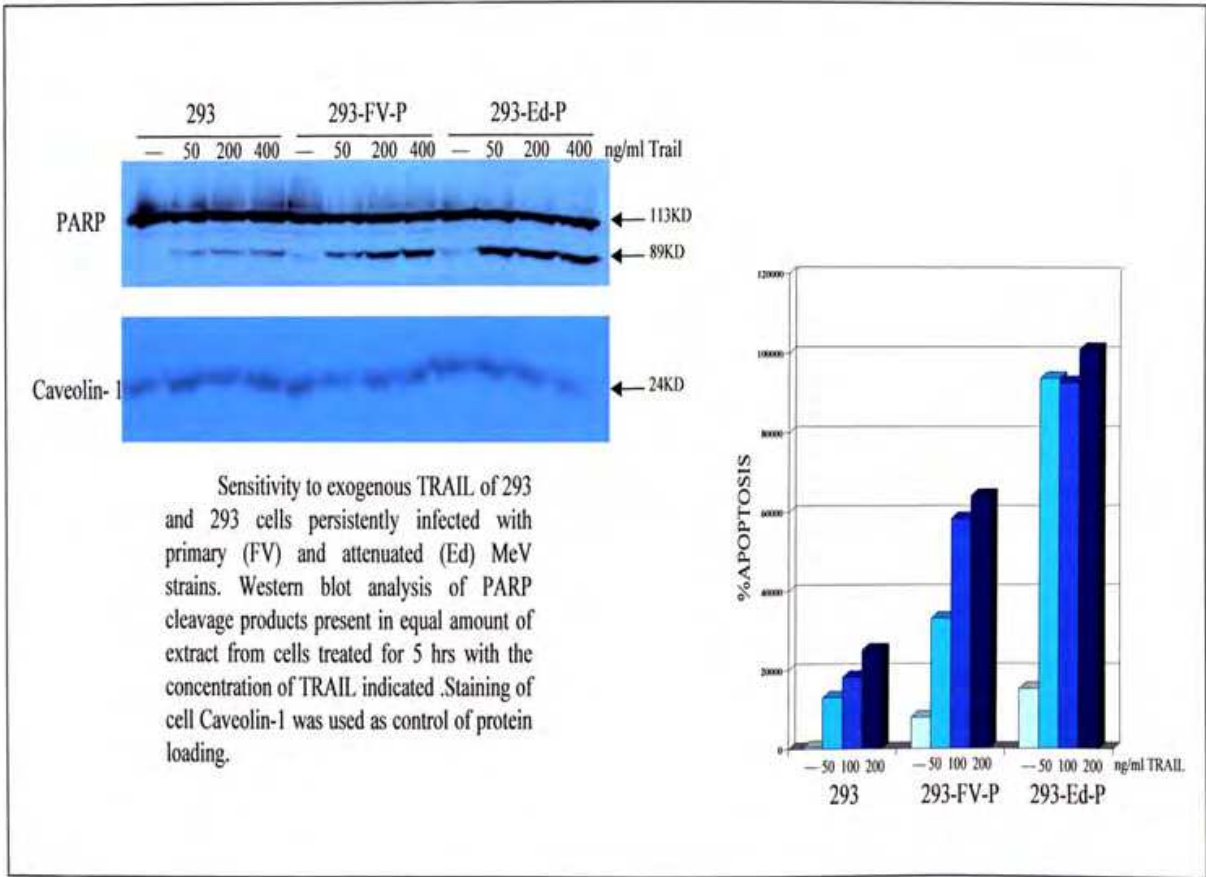


Fig. 9. Apoptosis in MV persistently infected cells treated with recombinant TRAIL.

Left panel. To measure the sensitivity of cells to recombinant TRAIL (tumour-necrosis-factor-related apoptosis inducing ligand) the expression of the uncleaved poly (ADP-ribose) polymerase (PARP)(113KD) substrate for apoptosis specific ICE-family proteases, and its cleaved product 89KD fragment were used as specific markers for apoptosis. Cells were untreated or treated with soluble human recombinant TRAIL with cross-linking enhancer or the killer TRAIL (His-tag) from Alexis, at concentrations and time indicated. Western blot analysis were performed in RIPA-cell extracts, normalized for protein concentration, run in SDS-PAGE , transferred to membranes end developed with specific antibodies. Staining of cell caveolin was used as control of protein loading.

293 cells were less sensitive to PARP cleavage induced by TRAIL than their persistent infected cell lines established with a vaccine strain or with a primary isolate. These finding in epithelial cells were further extended to human lymphoid B (Dakiki) and T (MOLT3) cells.

Right panel. To study the sensitivity to exogenous TRAIL of 293 and 293 cells persistently infected with primary (FV) and attenuated (Ed) MV strains, cell apoptosis was stimated as chromatine condensation in more than 200 nuclei after acridine orange staining. The specificity of acridine orange assay has been previously established in MV infected cells by DNA fragmentation detection techniques.

6. Conclusions

The comparison of the MV genomic sequence corresponding to Madrid SSPE cases SMa79, SMa84, and SMa94 with those of MV genotypes circulating in Madrid during the last 5 decades provided the first confirmation that the MV causing SSPE corresponds to the virus producing the measles acute infection and not to a possible re-infection years later at onset of the encephalitis. This was the first example of a human persistent infection by an RNA virus.

2. Concerning the question of where the virus could persist and replicate during the long latent period, we have observed that at least in the final stages of SSPE, MV is also present in abdominal and thoracic lymph nodes. The comparison of MV genomic RNA from brain and lymph nodes for each patient showed both viruses belong to the same genotype.

3. In two SSPE cases, those presenting an average and long disease course, but not in the short disease course case, biased hypermutation U to C was observed in the matrix M gene at a high level (38% U to C) and low level (10%U to C) respectively. The mutation map across the entire genome was the same from distant parts of each brain, supporting the indication of clonal origin of MV brain invasion proposed by V. ter Meulen, M. Billeter and collaborators. After the first description of biased U to C hypermutation phenomena by M.Billeter and collaborators in one brain from a MIBE case, our results were the first description of biased hypermutation in SSPE brain. Our results indicate that biased hypermutation U to C are found at autopsy in brain of SSPE patients after years of disease, and it is not proportional to the length of the disease. Biased hypermutation U to C is present in MV localized in lymph nodes at similar or higher level than in the respective brain, suggesting that biased hypermutation may take place also in infected lymphoid cells. In the three cases the transcription of M, F and H genes were down-regulated, and M protein ability to bind to MV nucleocapsids was impaired by deletion or biased hypermutation.

4. The length of MV genome found in the brain of SSPE remains constant, 15894 after years to decades of persistent infection, and no evidence of significant proportion of nucleocapsid subgenomic RNAs was found in the brains of the SSPE cases studied. Copy-back subgenomic RNAs were found in MV nucleocapsids only in one of the three brains, indicating that the presence of MV defective interfering particles is not an universal feature in SSPE.

5. Currently, no efficient treatment for SSPE or MIBE patients is available. New approaches to therapy of these lethal encephalitis are underway in several laboratories, and possibly the future treatments will combine several therapies to control MV infection by specific antiviral designed drugs and molecules that would counteract virus escape to host immune response. Today, the only effective way to prevent MV encephalitis is the implementation of measles vaccination programs.

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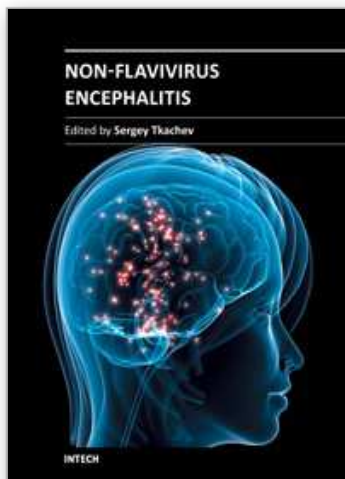
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This book covers the different aspects of non-flavivirus encephalitis of different etiology. The first section of the book considers general problems of epidemiology such as study of zoonotic and animal vectors of encephalitis causative agents and methods and approaches for encephalitis zoonoses investigations. The members of different virus species are known to be the causative agents of encephalitis, so the second section of the book is devoted to these viral pathogens, their epidemiology, pathology, diagnostics and molecular mechanisms of encephalitis development by such viruses as HIV/SIV, herpes simplex virus type 1 and equine herpesvirus 9, measles virus, coronaviruses, alphaviruses and rabies virus. The next section of the book concerns the study of protozoan pathogens such as toxoplasma and amoebae. The last section of the book is devoted to multicellular pathogen as human *Filaria Loa Loa* - a filarial worm restricted to the West Africa.

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