Virus Research 211 (2016) 29–37

Contents lists available at ScienceDirect

Virus Research

journal homepage: www.elsevier.com/locate/virusres

The phosphoprotein genes of measles viruses from subacute sclerosing panencephalitis cases encode functional as well as non-functional proteins and display reduced editing

E.L. Millar[a], L.J. Rennick[a,b], B. Weissbrich[c], J. Schneider-Schaulies[c], W.P. Duprex[a,b], B.K. Rima[a,*]

[a] Centre for Infection and Immunity, School of Medicine, Dentistry and Biomedical Sciences, The Queen's University of Belfast, Belfast, BT9 7BL Northern Ireland, UK
[b] Department of Microbiology, Boston University School of Medicine and National Emerging Infectious Diseases Laboratories, Boston University, 620 Albany Street, Boston, MA 02118, United States
[c] Institute for Virology and Immunobiology, University of Würzburg, Versbacher Straße 7, D-97078 Würzburg, Germany

ARTICLE INFO

Article history:
Received 2 July 2015
Received in revised form
22 September 2015
Accepted 23 September 2015
Available online 28 September 2015

Keywords:
Measles virus
Subacute sclerosing panencephalitis
Phosphoprotein gene
Editing
Genetic variation
Absence of biased hypermutation

ABSTRACT

Products expressed from the second (P/V/C) gene are important in replication and abrogating innate immune responses during acute measles virus (MV) infection. Thirteen clone sets were derived from the P/V/C genes of measles virus (MV) RNA extracted from brains of a unique collection of seven cases of subacute sclerosing panencephalitis (SSPE) caused by persistent MV in the central nervous system (CNS). Whether these functions are fully maintained when MV replicates in the CNS has not been previously determined. Co-transcriptional editing of the P mRNAs by non-template insertion of guanine (G) nucleotides, which generates mRNAs encoding the viral V protein, occurs much less frequently (9%) in the SSPE derived samples than during the acute infection (30–50%). Thus it is likely that less V protein, which is involved in combatting the innate immune response, is produced. The P genes in MV from SSPE cases were not altered by biased hypermutation but exhibited a high degree of variation within each case. Most but not all SSPE derived phospho-(P) proteins were functional in mini genome replication/transcription assays. An eight amino acid truncation of the carboxyl-terminus made the P protein non-functional while the insertion of an additional glycine residue by insertion of G nucleotides at the editing site had no effect on protein function.

1. Introduction

Measles, caused by measles virus (MV), is one of the most contagious human diseases known. Despite being a vaccine-preventable disease 122,000 deaths were attributed to MV infection worldwide in 2012 (Perry et al., 2014). Generally, infection results in an acute, self-limiting illness but serious complications can include bronchiolitis, pneumonitis and transient immune suppression that may lead to or aggravate previously acquired secondary infections. One rare but usually fatal neurological complication is subacute sclerosing panencephalitis (SSPE). Originally this was described to occur with a very low frequency (1:100,000), but more recent studies have estimated this to be 22:100,000 (Bellini et al., 2005) and in the most recent careful analysis with a good estimate of the denominator the frequency of SSPE was estimated to be between 1:1700 and 1:3300 in children that acquire measles under the age of 5. This approaches that of mortality associated with acute measles (Schönberger et al., 2013). SSPE is a progressive neurodegenerative disease which presents as loss of attention leading to ataxia and coma, and eventually death within months but sometimes up to several years. It takes an average of eight years between initial infection and onset of SSPE symptoms, and at present what triggers onset of clinical disease is not understood. Pathologically, SSPE is seen as severe demyelination, neuronal infection and progressive infection of non-neuronal cells in the central nervous system (CNS). However, even though there is an abundance of viral antigen in the brain in most cases, no infectious virus can be isolated from autopsy material, although co-cultivation allows propagation of syncytium forming, cell associated virus (Ogura et al., 1997). Currently, no treatments have been licensed to inhibit SSPE development or pro-

* Corresponding author.
E-mail address: b.rima@qub.ac.uk (B.K. Rima).

http://dx.doi.org/10.1016/j.virusres.2015.09.016
0168-1702/

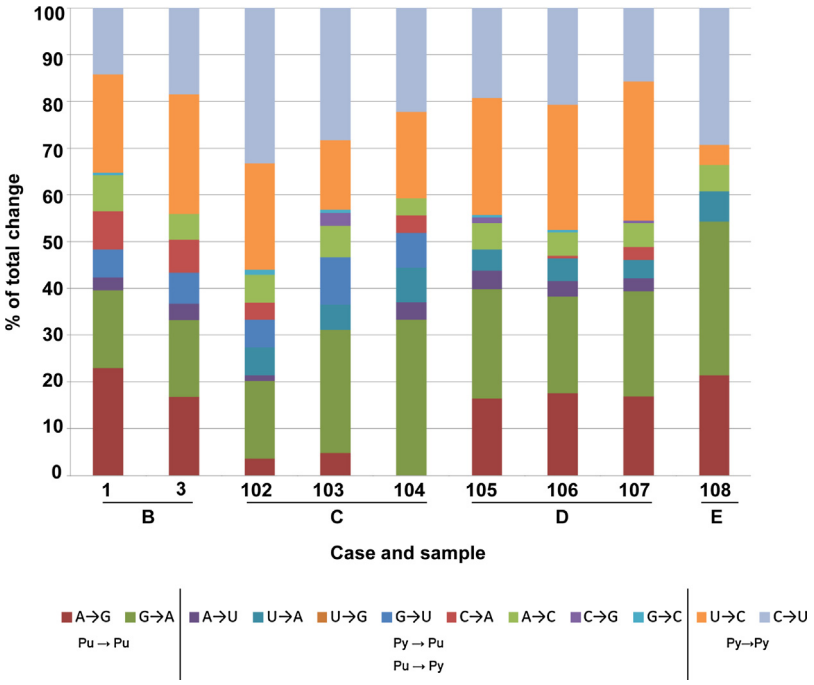

Fig. 1. Comparison of SSPE derived clone sequences to genotype reference strains does not show hypermutation.
Graph of percentages of nucleotide change types making up total change from reference strain for case B–E SSPE samples. Nucleotide changes are divided into pyrimidine to pyrimidine changes (top two bands), purine to purine changes (bottom two bands) and changes between pyrimidines and purines (middle bands).

gression. Although SSPE is caused by MV replication in the brain, the anatomical site of persistence of the virus between the acute infection of the patient and the onset of symptoms is unknown. Genotyping studies have shown that SSPE-derived MV sequences are strongly related to virus circulating at the time of initial infection rather than at the time of presentation (Jin et al., 2002; Rima et al., 1995). Hence it is clear that the original infecting virus persists and that SSPE is a paradigm for long-term persistence of RNA viruses. No general mechanistic model has been established for the control of persistence, but key alterations to the virus genome, replication cycle and host cell have been associated with driving MV to an intracellular existence in vivo, as reviewed by Rima and Duprex (2005). MV virions bind the cellular receptors CD150, also sometimes referred to as signaling lymphocyte activation molecule (SLAM/F1), PVRL4 (nectin-4), and CD46 (tissue culture adapted strains), but a receptor on brain cells has not yet been characterized (Mühlebach et al., 2011; Noyce et al., 2011; Rima and Duprex 2011). Interaction of a receptor with the hemagglutinin (H) glycoprotein activates the fusion (F) glycoprotein to direct fusion of virus envelope and cell plasma membranes. The incoming ribonucleoprotein (RNP) complex is composed of the 15,894 nucleotide negative sensed genomic RNA molecule and 2649 copies of the nucleocapsid (N) protein together with the P and large (L) proteins which comprise the viral RNA dependent RNA polymerase (RdRp). The RNPs are coated with the matrix (M) protein (Liljeroos et al., 2011). Expression of the six transcription units (N, P/V/C, M, F, H and L) by the RdRp proceeds by a stop-start mechanism from the genomic 3' terminus leading to a well-characterized transcription gradient. This gradient has been shown to be steeper in SSPE than in acute infection of tissue cultured cells (Cattaneo et al., 1987; Schneider-Schaulies et al., 1989), which leads to a reduction in SSPE in the amounts of the F, H and L proteins produced.

The MV genomes present in the brains of SSPE patients have well characterized mutations including biased U → C and A → G hypermutation in the genes encoding the M and H proteins (Cattaneo and Rose 1993; Cattaneo et al., 1988, 1989a; Wong et al., 1989) and premature stop codons affecting the cytoplasmic tail of the F glycoprotein (Schmid et al., 1992).

Little is known about how the V and C proteins might contribute to viral persistence in SSPE cases. The P protein is produced from the unedited P/V/C gene transcript, while V is produced from a transcript generated by co-transcriptional addition of a single pseudo-templated G nucleotide at the editing site (nucleotide 751). In acutely infected cells these insertions occur in approximately 50% of P mRNA molecules (Cattaneo et al., 1989b; Liston and Briedis 1995; Vidal et al., 1990) and are mediated via a RdRp stuttering mechanism; the insertion changes the open reading frame (ORF) giving access to the −1 frame at the editing site. The third protein expressed from the P/V/C gene, the C protein, is produced following initiation of translation at an alternative initiation codon allowing access to an ORF which overlaps P or V (Bellini et al., 1994). The C protein is encoded by both P and V mRNAs and has been shown to affect replication and transcription of measles virus as well as potentially affects translation by counteracting eIF2α phosphorylation. The V protein interferes directly in JAK/STAT signaling as well as sequestering mda5 and inhibiting activation of transcription factors NFκB and IRF7 (reviewed in Rima and Duprex, 2011) Given the importance of the V and C proteins in abrogating innate immune responses and the transcription/replication regulation we addressed the question of their genetic variability and function, especially in those viruses which establish long term persistent infection. Most known variations in the MV P protein occur in the amino terminal half of the protein, while the remainder of the protein is conserved. The C protein varies primarily in its amino terminus whilst the carboxy terminal amino acid residues of the V protein, which are unique to this protein, do not vary significantly between MV strains (Baczko et al., 1992). The ORFs for the N and L proteins are well conserved in SSPE virus sequences indicating their essential role in the replication of the virus (Baczko et al., 1992; Komase et al., 1995).

In this study we investigated to what extent MV in SSPE tolerates the fixation of mutations, caused by biased hypermutation or otherwise in the P/V/C gene and determined changes in the edit-