Comparative Immunology, Microbiology and Infectious Diseases 47 (2016) 7–18

Contents lists available at ScienceDirect

Comparative Immunology, Microbiology and Infectious Diseases

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

Review

Evolutionary characteristics of morbilliviruses during serial passages *in vitro*: Gradual attenuation of virus virulence

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ARTICLE INFO

Article history:
Received 30 January 2016
Received in revised form 22 May 2016
Accepted 24 May 2016

Keywords:
Morbilliviruses
Virulence attenuation
Serial passages
Quasispecies
Fitness
Mutation

ABSTRACT

The genus *Morbillivirus* is classified into the family *Paramyxoviridae*, and is composed of 6 members, namely measles virus (MV), rinderpest virus (RPV), peste-des-petits-ruminants virus (PPRV), canine distemper virus (CDV), phocine distemper virus (PDV) and cetacean morbillivirus (CeMV). The MV, RPV, PPRV and CDV have been successfully attenuated through their serial passages *in vitro* for the production of live vaccines. It has been demonstrated that the morbilliviral virulence in animals was progressively attenuated with their consecutive passages *in vitro*. However, only a few reports were involved in explanation of an attenuation-related mechanism on them until many years after the establishment of a quasispecies theory. RNA virus quasispecies arise from rapid evolution of viruses with high mutation rate during genomic replication, and play an important role in gradual loss of viral virulence by serial passages. Here, we overviewed the development of live-attenuated vaccine strains against morbilliviruses by consecutive passages *in vitro*, and further discussed a related mechanism concerning the relationship between virulence attenuation and viral evolution.

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http://dx.doi.org/10.1016/j.cimid.2016.05.007
0147-9571/

1. Introduction

The genus *Morbillivirus* is classified into the sub-family *Paramyxovirinae*, family *Paramyxoviridae*, order *Mononegavirales* in the latest taxonomy of the International Committee on Taxonomy of Viruses. This genus contains 6 viruses, namely measles virus (MV), rinderpest virus (RPV), peste-des-petits-ruminants virus (PPRV), canine distemper virus (CDV), phocine distemper virus (PDV) and cetacean morbillivirus (CeMV). They are highly infectious, spread via the respiratory route, cause profound immune suppression [1], and if non-attenuated in virulence, would correspondingly cause devastating diseases of humans and animals.

Fortunately, the most important viruses (MV, RPV, PPRV and CDV) in this genus have been successfully attenuated for the production of live vaccines to reduce their impacts on human and animal health. However, only a few reports could be found to illustrate a live-attenuated mechanism when morbilliviruses were serially passaged in cells. Thus, we critically reviewed the evolutionary dynamics related to virulence attenuation of morbilliviruses *in vivo*, after a historical overview of the development of live-attenuated vaccine strains against them.

2. General characteristics of morbilliviruses

2.1. Virion structure

Morbilliviruses are pleomorphic particles (Fig. 1) with lipid envelope enclosing a ribonucleoprotein core that contains the genome, a single strand of RNA with negative polarity [2]. Their genomes encode for six structural proteins in the order of nucleocapsid (N) protein, phosphoprotein (P), matrix (M) protein, fusion (F) protein, hemagglutinin (H) protein and large (L) protein (Fig. 1). The P gene encodes for two non-structural proteins, the V and C proteins. The PPRV has a genome length of 15,948 [3,4] or more recently even 15,954 [5] nucleotides (nts), which is considered the longest one in the genus *Morbillivirus*.

The genome is surrounded by the N protein to form a nucleocapsid, where the P and L proteins are coupled also. The L protein is the viral RNA-dependent RNA polymerase (RdRp), for which the P protein acts as a co-factor. The M protein underneath the lipid envelope acts as a link, which associates the nucleocapsid with the F and H proteins. Moreover, the M protein, binding to cytoplasmic tails of the H and F proteins [6], appears to play a complex role in the assembly and budding of virion, and meanwhile is required to downregulate H/F protein-mediated fusion between infected and neighboring uninfected cells [7].

2.2. Virus life cycle

A signaling lymphocyte activation molecule (SLAM, also known as CD150) has been proven to act as a common receptor for the MV [8], RPV [9], CDV [10], PPRV [11] and marine mammal morbilliviruses [12]. The morbilliviral H protein attaching to the SLAM receptor is the first step of viral replication cycle, followed by the F protein-mediated fusion between viral envelope and cellular membrane, causing release of the nucleocapsid into a cell. Subsequently, a series of biochemical reactions, including nucleocapsid uncoating, genome replication, mRNA transcription, protein expression, genome encapsidation and progeny budding, sequentially occur in the cell to finish finally a life cycle, as shown in Fig. 2. The progeny virions have an ability to infect neighboring cells, and then maintain uninterrupted life cycles.

2.3. Phylogenetic relationship

Many members of the genus *Morbillivirus* share a high antigenic relatedness [13,14] and genetic homology [15,16] with each other, demonstrating that they are descended from a common ancestor. It has been proposed that the RPV is the archevirus of morbilliviruses, from which the CDV is first to evolve, followed by the MV [17]. The PPRV was once considered as a variant of the RPV until a new classification reported in the late 1970s [18]. A phylogenetic tree (Fig. 3) of these 6 morbilliviruses was constructed here based on their complete sequences of the N gene from the Genbank. The phylogenetic analysis revealed that the MV, RPV, PPRV and CeMV shared a higher homology with each other than with the CDV and PDV, both of which were clustered into another evolutionarily similar lineage.

2.4. Species susceptibility

Each mainly susceptible animal was illustrated correspondingly in its viral lineage in Fig. 3. The MV is the pathogen of measles, an infection of the respiratory system in humans and many nonhuman primates, and evolves from the formerly widespread RPV [19], which mainly infects cattle and some other species of even-toed ungulates. Fortunately, the RPV had been eradicated worldwide through human intervention [20]. The PPRV primarily affects goats and sheep, occasionally infecting wild small ruminants [21], buffalos, camels and even pigs [22]. The CDV affects a wide variety of animal families, including dogs, coyotes, foxes and so on [23], and is most closely related to the PDV, which caused two mass mortalities of seals in Europe in 1988 [24] and 2002 [25], respectively. In addition to the seals, several cetacean species can also be infected by other morbilliviruses (CeMV), including the porpoise morbillivirus (PMV) [26] and dolphin morbillivirus (DMV) [27].

2.5. Immune responses

The immune responses against morbilliviruses in their hosts are associated with the hosts' innate and adaptive immune systems. It has been repeatedly reported that the MV [28], PPRV [29], RPV [30] and CDV [31] can induce severe immunosuppression in their hosts. For example, an analysis on MV-infected cases showed a significant decrease in total lymphocyte count, due mainly to a decrease in helper/inducer T lymphocytes [32]. Many mechanisms underlying morbillivirus-induced immunosuppression have been proposed, such as inhibition of leukocyte proliferation [33,34] and depletion of uninfected lymphocytes [35]. The N protein has been proven to not only be incapable of eliciting a neutralizing antibody response, but also be effective in inhibiting an inflammatory immune response in mice by binding a murine Fcγ receptor [36].