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Measles: What we have learned from non-human primate models

Rik L. de Swart

Department of Viroscience, Postgraduate School of Molecular Medicine, Erasmus MC – University Medical Center, P.O. Box 2040, 3000 CA Rotterdam, The Netherlands

Studies in non-human primates (NHPs) have been crucial for our understanding of measles as a high impact viral disease of humans. Over a century ago, inoculations of NHPs with filtered secretions from measles patients first identified a virus as the causative agent of this disease. In the 1960s, studies in NHPs with measles virus isolates passaged *in vitro* provided the basis for live-attenuated measles virus vaccines, which became one of the most successful medical interventions in history. More recently, experimental infections of NHPs have provided critical contributions to our understanding of the tropism and pathogenesis of measles virus. This review briefly highlights some of the lessons learned from NHP models of measles virus infection.

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Measles

Before the introduction of vaccination, measles was a common infectious disease that caused frequent outbreaks associated with high morbidity and mortality [1,2]. Measles usually manifests itself as childhood disease in densely populated areas, but non-immune humans of all ages can develop the disease [3]. The causative agent, measles virus (MeV), is one of the most contagious pathogens of humans, and is spread by airborne transmission [4]. The virus is a member of the family *Paramyxoviridae*, genus *Morbillivirus* and contains a non-segmented single stranded RNA genome of negative polarity, encapsidated by the viral nucleoprotein and surrounded by a lipid envelope. Measles has an incubation period of approximately two weeks, after which patients develop cough, fever and a typical maculopapular skin rash. However, hallmark of measles is the transient but severe immune suppression that develops during the resolution of disease and can persist for over two years, resulting in increased susceptibility to opportunistic infections [5,6]. This often leads to complications such as pneumonia or severe diarrhea, which are a major cause of measles-associated mortality. Introduction of safe and effective live-attenuated measles virus vaccines has strongly reduced global childhood mortality [6]. Indeed, development of today's MeV vaccines has depended strongly on NHP studies [7,8]. However, MeV is still estimated to cause almost a hundred thousand deaths each year, all of which are vaccine-preventable [9].

Measles pathogenesis

Although MeV is transmitted via the respiratory route, its pathogenesis differs from most other respiratory viruses. The virus causes a systemic disease, involving infection of various cell types mediated by at least two cellular receptors. In 2000, CD150 (or SLAM-F1) was identified as a cellular receptor for

E-mail address: (r.deswart@erasmusmc.nl)

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wild-type MeV [10], followed by the identification of nectin-4 (or PVRL4) as a second cellular receptor [11,12]. CD150 is expressed by subsets of myeloid and lymphoid immune cells. Nectin-4 is an adherens junction protein that is expressed by keratinocytes and differentiated respiratory epithelial cells, but exclusively at their basolateral side. In addition, it has been shown that the C-type lectins DC-SIGN and Langerin, expressed by dendritic cells (DCs) and Langerhans cells, can serve as attachment receptors for MeV. Although these do not mediate viral entry, they strongly enhance virus transmission from DCs to CD150-positive lymphocytes [13,14]. It is now thought that MeV enters the host by initial infection of myeloid cells (macrophages and DCs) in the respiratory tract, which serve as ''Trojan horses'' to transmit MeV to CD150-positive T- and B-lymphocytes in the draining lymphoid tissues. These MeV-infected lymphocytes subsequently mediate a cell-associated viremia, resulting in dissemination to virtually all peripheral lymphoid tissues, including local DCs and lymphocytes in the submucosa of the respiratory epithelium. These cells transmit the virus to respiratory epithelial cells through basolateral infection, mediated by nectin-4. Infection of epithelial cells results in apical budding of novel virus particles, concurrent with epithelial damage that induces cough, which in turn supports airborne transmission of MeV to the next host [15].

Measles animal models

A variety of animal species has been considered as model of MeV infection. Small laboratory animals are in most cases not susceptible to MeV infection, with the exception of cotton rats [16]. However, MeV infection of cotton rats does not recapitulate the complex pathogenesis of measles as seen in humans. Several transgenic rodent models have been developed, but these required both expression of MeV receptors and knockout of type 1 interferon responses to support MeV entry and replication [17]. Therefore, two options remain: infections with animal morbilliviruses in their natural host species (e.g. canine distemper virus infection of ferrets [18,19]) or experimental infection of NHPs with MeV [8,20]. Whereas canine distemper virus was first identified in dogs, it can infect virtually all carnivores. Similarly, MeV should be considered as a virus that infects primates rather than a virus that exclusively infects humans. However, due to the fact that MeV exclusively infects previously unexposed primates, the virus can only maintain endemic circulation in populations of more than 250,000 individuals [21]. As free-ranging NHP populations are well below this size, MeV does not circulate among NHPs under natural conditions but exclusively causes outbreaks after contact of NHPs with humans [8]. Experimental infections of NHPs have been essential for our understanding of the tropism of both wild-type and vaccine MeV strains, the pathogenesis of measles and measles immune suppression, and the efficacy of MeV as vaccine vector or oncolytic virus.

Measles virus tropism in NHPs

The incubation period between MeV infection and onset of skin rash is on average two weeks [4]. Therefore, studying early aspects of the pathogenesis of measles in humans has been difficult. The development of recombinant MeV strains that express luminescent or fluorescent proteins, combined with the large spectrum of antibodies available for specific phenotyping NHP cell types by flow cytometry and/or histology, has enabled accurate tropism studies in NHPs that elucidated MeV entry, pathogenesis, dissemination and immune suppression (as outlined in paragraph 2 of this review) [20,22–27]. In addition, generation of so called ''receptor-blind'' recombinant viruses, unable to bind to either CD150 or nectin-4, has helped elucidate the roles of both cellular entry receptors in NHPs [28–30]. More recently, tropism studies were conducted with the live-attenuated MeV vaccine strain Edmonston-Zagreb expressing green fluorescent protein, which was found to predominantly target myeloid antigen-presenting cells in the muscle of NHPs after intramuscular injection [31]. This recombinant vaccine virus was also used to study tropism and immunogenicity upon respiratory vaccination, demonstrating that the vaccine virus needed to be delivered to the lower respiratory tract for optimal immunogenicity and induction of protective immunity [32].

Measles immune suppression in NHPs

As described above, one of the most important sequelae of measles is immune suppression, significantly contributing to measles morbidity and mortality. Interestingly, measles is associated with simultaneous immune suppression and immune activation [33]: despite the fact that MeV causes lymphopenia and reduces host resistance to other infections, it also induces a strong immune response to itself resulting in life-long protection from measles. This phenomenon is commonly referred to as the ''measles paradox''. Studies of MeV infection in NHPs elucidated a tropism for specific lymphocyte subsets, which resulted in a novel model for measles immune suppression that is fully compatible with the measles paradox. Infection of specific CD150-positive lymphocyte subsets resulted in a partial depletion of memory lymphocyte subsets, leading to ''immune amnesia''. This depletion is masked by the rapid proliferation of MeV-specific lymphocytes, which mediate clearance of MeV-infected cells but cannot protect the host from other infections [34,35]. This model also explains the fact that measles immune suppression can last for several weeks to months after measles, whereas measles lymphopenia is usually resolved within a week after recovery. Additional support for this model was obtained from epidemiological studies in humans, that

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suggested that measles immune suppression may extend over a period of more than two years [6]. Whether or not the prolonged persistence of MeV RNA in lymphocytes, another phenomenon which has been detected in NHPs, contributes to long-term measles sequelae remains to be determined [36,37].

Measles vaccination in NHPs

In addition to elucidating the tropism of the existing live-attenuated MeV vaccine, NHPs have also been used to evaluate new generation MeV vaccines and novel routes of MeV vaccination [38,39]. In addition to needle-free vaccination via the respiratory route [40,41] (referred to in paragraph 4), another promising alternative for the use of hypodermic needles to deliver the live-attenuated MeV vaccine are microneedle patches [42,43]. These micron-scale dissolvable polymeric needles were formulated to encapsulate the standard live-attenuated MeV, and were used for intradermal vaccination without the requirement of vaccine reconstitution. Studies in NHPs have provided proof of principle of this approach, supporting further clinical development.

It is important to note that live-attenuated MeV strains are not only used for measles vaccination, but are also being developed as recombinant vectors for delivery of other antigens [44]. Several candidates have been evaluated in NHPs, including MeV-based candidate vaccines against Epstein–Barr virus, hepatitis B virus, HIV-1, human papilloma virus, Nipah virus and West Nile virus, some of which have progressed to clinical testing in humans (Chikungunya virus and Zika virus [45,46]).

Measles-based oncolytic virotherapy

In addition to playing a role as vaccine vector, an alternative application of live-attenuated MeV vaccines that has emerged relatively recently is oncolytic virotherapy [47,48]. The majority of these studies is performed in mouse models engrafted with human tumours, which means that the virus usually has limited potential to infect the host and mainly targets the tumour. Although in this arena efficacy studies are not likely to be performed in NHPs, safety studies may be of crucial importance for further development of these strategies [49].

Replacement, reduction and refinement

Implementation of the principles of the 3Rs (replacement, reduction and refinement) is of crucial importance for all studies in which animal models are considered, but may be even more critical when considering NHP studies. Many aspects of virus tropism and replication can be studied *in vitro*, using either immortalized cell lines, primary cells, organoids or *ex vivo* tissue culture. However, as described above morbilliviruses have a complex pathogenesis, which involves many organs and cell types and directly interferes with the host immune response. Therefore, complete replacement of the use of animals will be impossible. Reduction strategies aim to minimize the number of animals per experiment, which can be achieved by improving study design and focusing on the robustness and reproducibility of the experiments. The use of recombinant viruses that express fluorescent reporter proteins has strongly contributed to this goal. Finally, refinement (improving animal welfare) is of crucial importance. This involves the best possible housing and care, and implementation of optimal *in vivo* technologies, including adequate anesthesia and analgesia. These strategies are further supported by the fact that an increasing number of scientific journals required studies to be compliant with the ARRIVE guidelines for reporting of research using animals [50].

Concluding remarks

The scientific committee on Health, Environmental and Emerging Risks (SCHEER) has recently updated its opinion on ''The need for non-human primates in biomedical research, production and testing of products and devices'' [51]. The opinion focuses on approaches aimed at implementation of the 3Rs in studies that use NHPs. Although the ultimate objective remains phasing out the research use of NHPs in Europe, the Opinion concluded that NHPs remain indispensable for particular types of research for now. Among these are the study of measles pathogenesis and possible applications of recombinant MeV for innovative developments for prevention (vaccines against MeV and other agents) and therapeutics (e.g. oncolytic virotherapy).

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