Systemic spread of measles virus: Overcoming the epithelial and endothelial barriers

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Summary

As the major entry receptor, signalling lymphocytic activation molecule (SLAM, CD150) essentially determines the tropism of measles virus (MV) for immune cells. This receptor is of considerable importance for the induction of immunomodulation and suppression, and for the systemic spread of MV to organs including secondary lymphoid tissues, the skin, the respiratory tract, and the brain predominantly via infected cells of the immune system. But how does the virus cross the epithelial barrier during initiation of the infection, the blood organ barriers formed by endothelial cells, and the epithelial barrier from within, when virus will be released from the host? Additional unknown receptor(s) on CD150-negative epithelial and endothelial cells have been postulated. However, it has also been postulated (and demonstrated in macaques) that the initial infection is independent from usage of this receptor, and that the first target cells appear to be CD150-positive cells in the epithelium. For later stages of the infection, for virus release from the host, it is claimed that this unknown receptor on epithelial cells is required for crossing the barrier from within. The endothelial cell barrier must be crossed from the apical (luminal) to the basolateral (abluminal) side to carry the infection to organs and the skin. However, infected leukocytes are impaired in several functions including transmigration through endothelial cells. The infection may spread via cell contact-mediated infection of endothelial cells and basolateral virus release, or via migration of infected leukocytes.

Keywords

Viral infection, cerebrovascular disease, receptors

General aspects of measles

Measles is a well defined clinical disease normally contracted by children and young adults. In contrast to most other respiratory virus infections, infection with measles virus (MV) results in systemic disease. Clinical symptoms associated with measles are fever and rash, often accompanied with cough, coryza or conjunctivitis, but also a severe transient immunosuppression favouring the acquisition and aggravation of secondary infections which enhance the lethality of the disease. In spite of the implementation of an efficacious vaccine, more than 30 million cases of acute measles are still reported annually with approximately 200,000 fatalities (1), the majority of which develop in Third World countries as a direct consequence of immunosuppression (2). While a long lasting virus-specific immunity is efficiently induced during acute measles and after vaccination, there is a generalised transient suppression of immune responses to other infections lasting for several weeks. This feature of measles was noted decades before the first isolation and characterisation of MV, which was duly recognised as the first immunosuppressive pathogen. The term ‘anergy’ was coined by von Pirquet in 1908 (3) to describe the loss of delayed-type hypersensitivity (DTH) reactions to tuberculin in MV-infected individuals. Proliferative responses of lymphocytes to polyclonal and antigen-specific stimulation ex vivo are also highly impaired (4–6). In spite of the transient immunosuppression, in most cases of acute measles, infiltration of mononuclear cells into local areas of virus replication, the appearance of antiviral antibodies, and virus-specific T-cells in the blood mark the onset of a virus specific immune response. This immune response is accompanied by soluble CD4, CD8, IL-2R and β2 microglobulin in serum, and a Th1 cytokine profile that later switches to a Th2 type as indicated by a rise in IL-4 plasma levels (reviewed in [5]). Measles is associated with a pronounced leukopenia affecting B-cells, monocytes, neutrophils, as well as CD4+ and CD8+ T-cells, the extent of which seems to be related to the age-
dependent severity of the disease (7, 8). In contrast to B-cell numbers, which can still be below control levels for up to six weeks, numbers of T-cells return to normal after 10 days and the CD4/CD8 ratio remains constant over time (8–10). T-cell depletion could well occur in secondary lymphoid tissues by acquisition of MV or apoptotic signals from professional antigen-presenting cells. In support of this hypothesis, fusion or apoptosis was found to be induced in T-cell co-cultures with MV-infected dendritic cells (DCs) (11–15). However, the frequency of infected cells in the periphery is low during measles throughout all stages of the acute disease, so that at least for peripheral T-cells, infection-mediated loss probably does not substantially contribute to T-cell lymphopenia (4, 5). As an alternative mechanism for the loss of peripheral T-cells, disappearance of highly activated LFA-1+ T-cells (LFA, lymphocyte function associated antigen) possibly by disruption of recirculation and random homing has also been proposed (16).

After infection of an individual, MV has to first pass through the respiratory epithelium, and then is transported in infected CD150-positive DCs, or alveolar macrophages, to draining lymph nodes. Alternatively, CD150-positive lymphocytes in the tonsillar crypts may also be first target cells, which initiate infection of draining lymph nodes and the establishment of viraemia. From the blood stream MV must overcome endothelial barriers to reach organs and the skin. In the last phase of the acute infection, the virus must again pass through the respiratory epithelial barrier, however this time from the basal side, to spread to the next host by coughing and sneezing. While older text books refer to infection of epithelium in the initial and late phases of the disease, it was not clearly specified which cell types are actually infected. Similarly, it is also unknown how MV is able to overcome endothelial barriers. In rare cases of acute measles (1:10000–20000) (17), following a clinically silent period of years, MV may cause the inevitably fatal brain disease, subacute sclerosing panencephalitis (SSPE) (18). However, it is unknown when and by what route MV enters the central nervous system (CNS) though there is some circumstantial evidence that it has been adapted to growth on Vero cells. As a consequence of the cell surface interaction with the H protein, CD46 is downregulated from the cell surface which, unsurprisingly given the natural function of this molecule, has been associated with an enhanced sensitivity of MV-infected cells to complement mediated lysis (27, 28).

In contrast to this special property of attenuated MV strains, all MV strains (including lymphotropic wild-type viruses and attenuated viruses) are able to utilise CD150 (signalling lymphocytic activation molecule, SLAM), a member of the CD2 subset of the Ig superfamily, as an entry receptor (29–34). The basis for the restriction of wild-type MV-H proteins to functionally interact with CD46 has recently been documented following the resolution of the H protein structure (24). In corroboration of their similarities in cell tropism and pathogenicity, other morbilliviruses such as canine distemper virus (CDV) and rinderpest virus (RPV) also utilise the species specific homologues of CD150 as major cellular entry receptors (35, 36). CD150 is expressed on activated B-cells, activated and memory T-cells including activated regulatory T-cells (37), and immature thymocytes (38). In line with this expression pattern, CD150+B-cells represented the prime target-cell population in human tonsillar tissue material infected with MV wild-type strains in vitro (39). Within the T-cell compartment, MV infection clearly segregated with CD150 expressing CD45RO+ memory cells which were infected less frequently than B-cells, but appeared to be preferentially depleted from the infected tissue. Although the basis of this mechanism is undetermined, preferential infection-mediated depletion of T-cells in secondary lymphoid tissues might well contribute to peripheral T-cell lymphopenia. The preferential tropism of wild-type MV for B-cells (both in lymphoid tissues and in

Viral envelope proteins

Measles virus is the type species of the genus Morbillivirus, and member of the family Paramyxoviridae, and has a negative-stranded RNA genome which encodes six structural and two non-structural proteins. Functionally, morbilliviral structural proteins can be grouped into those essential for the replication of the viral genome: the nucleocapsid (N) protein and the polymerase complex consisting of the large polymerase protein (L) and its cofactor, the phosphoprotein (P), forming with the viral RNA the ribonucleoprotein particle (RNP)-complex, the basic matrix (M) protein which interacts with the RNP-complex and the envelope and those associated with the viral lipid bilayer membrane (the fusion (F) and haemagglutinin (H) proteins) forming the envelope. Viral entry relies on the interaction of the H protein with receptor molecules on the host cell surface (see below) followed by a pH-independent conformational change of the F protein, which inserts its fusogenic domain into the target cell membrane thereby initiating the membrane fusion process required for uptake of the viral core complex into the host cell cytoplasm. When expressed at the cell membrane of infected cells, the protein complex also causes fusion with adjacent uninfected cells, provided these express appropriate receptors for the H protein. This gives rise to typical syncytia both in tissue culture and to a lesser extent in vivo. Apparently, the fusion activity of the F/H protein complex is negatively regulated by the M protein, which physically and functionally interacts with the cytoplasmic domains of the F and H proteins (19, 20). In addition to controlling fusogenicity, the M protein is a driving force in promoting viral budding, but also acts as a negative regulator of morbilliviral transcription by mechanisms which remain to be elucidated (21, 22). New structural analyses of the MV-H protein (23, 24) have provided valuable insights into the location of receptor binding domains and neutralising antibody motifs. These studies demonstrated that the receptor-binding head domain forms a homodimer and exhibits a cubic-shaped β-propeller structure.

Cellular receptors for measles virus

The first molecule identified as a cellular receptor for MV was CD46 (membrane cofactor protein, MCP), a member of the complement regulatory proteins which is ubiquitously expressed on human nucleated cells (25, 26). However, high affinity binding to CD46 is confined to attenuated MV strains and isolates which have been adapted to growth on Vero cells. As a consequence of the cell surface interaction with the H protein, CD46 is downregulated from the cell surface which, unsurprisingly given the natural function of this molecule, has been associated with an enhanced sensitivity of MV-infected cells to complement mediated lysis (27, 28).

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Infections of the Endothelium

The tropism of MV during natural infection predominantly, but not completely, segregates with its usage of CD150 as a virus entry receptor, and it is unclear how the virus infects CD150 negative cells in vivo such as epithelial cells, endothelial cells, and in the case of infections with neurological involvement, neurons, oligodendrocytes, and astrocytes.

Moreover, in-vitro infections of primary endothelial cells (50), small airway epithelial cells (51), and a lung carcinoma epithelial cell line (52) by wild-type MV clearly occurred independently of CD150. In DCs, consequences of CD150 ligation either by antibodies or MV have not yet been addressed.

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Moreover, in-vitro infections of primary endothelial cells (50), small airway epithelial cells (51), and a lung carcinoma epithelial cell line (52) by wild-type MV clearly occurred independently of CD150. In the absence of CD150, the virus fails to establish productive infection even in primary human monocytes/macrophages (tissue resident or peripheral). The MV entry receptor on the surface of epithelial and endothelial cells remains to be identified but intriguing progress has been made in the study of the interaction of the H protein with this unknown protein. In a recent study, the generation and analysis of MV-H protein mutant plasmids resulted in the identification of three amino acids (L482, Y541, and Y543) critical for mediating fusion of NCI-H358 (human non-small bronchioloalveolar carcinoma of the lung, NCI-358) cells via interaction with the putative epithelial cell receptor (59). The importance of these residues was also verified in the context of the virus through the generation of an epithelial blind recombinant MV (60). Hydrophobic interactions may have a critical role in this interaction due to the presence of aromatic side chains on the identified amino acids in the potential receptor binding site which has been shown to be distinct from the putative CD46 and SLAM binding sites on the H protein. Indeed, the observation that residues Y541 and Y543 are conserved amongst morbilliviruses suggests that a common entry receptor may be present on epithelial cells derived from a number of different host species. It remains to be determined if these amino acids are also critical for the entry of wild-type MV into endothelial cells or other SLAM negative cells such as neurons, oligodendrocytes and astrocytes.

Leonard et al. (60) subsequently utilised an analogous strategy of analysing H protein mutant plasmids to show that amino acid P497 in addition to L482 and Y543 is a constituent of a novel receptor binding pocket on the H protein.

As already stated, in the early studies on natural measles, executed without human cellular markers, epithelial infection, predominantly of the respiratory system, was reported without stating the cell type which was infected. The growth in our knowledge of epithelial cellular complexity and the antigen-sensing role of intraepithelial immune cells has raised the possibility that the latter are the prime target for MV infection. This is not to say that epithelial cells cannot be infected by MV, but rather that epithelial cell infection is not an essential pre-requisite for measles pathogenesis. This hypothesis is supported by data from the recently reported monkey model (40) (see below). To further our knowledge of the human disease we undertook a molecular pathogenetic study in 19 cases of measles, 17 of which were fatal. The cellular pathological response was assessed against data on patient age, sex, country, year of presentation, phase in the course of measles, co-existing disease, cause of death and (with the exception of the nervous system) organ involved. Against all of these criteria the pathological response was consistent with predominant infection of cells of the immune system (unpublished).
Infection of CD150-positive cells as first target cells, spread of infection, and immunosuppression

Initial infection by morbilliviruses and subsequent virus spread are mainly determined by the presence of specific host cell receptors. Although MV infection doubtlessly occurs via the respiratory tract, primary target cells have not been identified. Epithelial cells are susceptible to infection with certain MV strains in vitro, but do not express the receptor for wild-type strains of MV, SLAM, which is restricted to the haematopoetic system. Instead, MV may be acquired by tissue resident macrophages or DCs within the epithelium and transported to local lymphatic tissues (Figure 1 A).

In fact, MV-infected MHC class II positive cells with DC morphology have recently been detected in peripheral mucosal tissues and isolated from skin explants of macaques experimentally infected with an EGFP-tagged recombinant wild-type MV (40). This study impressively confirmed the pronounced tropism of MV for the lymphatic system including lymph nodes, where the virus is believed to infect B and T cells and monocytes/macrophages, which subsequently mediate systemic virus spread by a cell-associated viremia. Thus, MV can be re-isolated from human peripheral blood lymphocytes (which is greatly enhanced by mitogen stimulation) and MV-specific RNA and proteins are detectable in a small proportion of peripheral lymphocytes and monocytes during and for few days after the rash (41, 61, 62). Although figures vary depending on the method used for detection, the overall percentage of infected peripheral blood mononuclear cells does not exceed 2% at any stage of infection and similar frequencies have recently been determined in experimentally infected macaques (40). Interestingly, in contrast to measles, CDV-infection (of ferrets) leads to a high percentage of infection of peripheral blood lymphocytes (up to 40% of T and 60% of B-cells) by day 7 post-infection (63).

Infection of macaques with a recombinant wild-type MV expressing EGFP combined a tool which enabled virus detection with unprecedented sensitivity and the most suitable animal model (40). At the peak of viraemia, EGFP fluorescence was detected in skin and the respiratory and digestive tract, but highest percentages of infected cells (up to 30%) were found in secondary lymphoid organs. In peripheral tissues, large numbers of MV-infected myeloid DCs were detected in conjunction with infected T-cells, suggesting transmission of MV between these cell types (40). Further analysis of the respiratory tract of macaques infected with fluorescent MV strains at earlier time-points post-infection may help to confirm the role of pulmonary DCs in the initial stages of measles. Another animal model system in which MV infection can occur via the respiratory tract are cotton rats (Sigmodon hispidus). In these animals, the H-protein dominated tropism of the virus directly reflects virulence of wild-type virus in humans by enabling the spread of recombinant viruses expressing wild-type H-proteins to draining lymph nodes, and immunosuppression as evidenced by impaired proliferation of lymphocytes ex vivo (64–66). However, analysis of DC involvement in this model has not been possible.

Figure 1: Possible strategies utilised by MV to cross the epithelium of the lung (A) and endothelial barriers (B). A) In the standard 'textbook' model, ciliated bronchial epithelial cells lining the respiratory tract are infected by MV via the apical surface. Spread of the virus to underlying dendritic cells results in amplification of the infection in a regional lymph node. b) MV infection of SLAM positive pulmonary dendritic cells via cellular processes which are exposed to the bronchial lumen in the absence of epithelial cell infection. c) Damage to the outer layer of ciliated epithelial cells results in an increased susceptibility of epithelial cells to MV infection via the exposed basolateral surface. Subsequent spread of virus through epithelial cell layers to underlying dendritic cells results in systemic MV spread via infection of a regional lymph node. d) Following systemic spread of MV, fusion between an infected dendritic cell or lymphocyte and the basolateral surface of an epithelial cell results in a focus of infected epithelial cells and apical virus release. B) Possible ways of MV to cross endothelial cell layers of blood vessels. a) Transmigration of infected leukocytes, and b) infection of endothelial cells and virus release into underlying tissue.
In immunocompetent mice transgenic for human CD150 (expression of which was driven by the CD11c promoter) MV antigens were detected in a very limited number of splenic CD11c+ DCs after intravenous infection by MV wild-type (67). Due to the restricted expression pattern of the transgene, DCs and a limited number of macrophages were the only susceptible cell types the virus could infect, and thus potential infection of other cell types (found to express CD150 in humans) could not be evaluated. Interaction of MV with CD150 on these cells reduces their capacity to synthesise IL-12 in response to TLR4 agonists (68). Thus, the vast majority of findings related to the role of DCs in MV pathogenesis and immunomodulation rely on in-vitro findings obtained in pure DC or DC/T-cell co-cultures.

Overcoming epithelial and endothelial barriers

Recently, a number of studies (58–60) have together contributed to a reassessment of the standard textbook model of measles, which hypothesised that MV gained entry into the body via infection of the apical surface of epithelial cells lining the respiratory tract. These studies have demonstrated that the apical surface of epithelial cells is resistant to MV infection and instead support a role for pulmonary DCs in the initial stages of measles. The role of epithelial cells in the transmission of MV in late stages of the infection has been investigated by Leonard et al. (60), who reported that a recombinant MV unable to bind to the putative epithelial cell receptor remains virulent in macaques but is unable to cross the airway epithelium and is not shed. However, further investigation of the properties of epithelial-blind MV in vivo is warranted due to the lack of any histopathological analysis of tissues from these animals to confirm the absence of epithelial cell infection. It is perhaps also surprising that a total absence of infectious virus was reported in broncho-alveolar lavage (BAL) samples taken from macaques, as de Swart et al. (40) have previously reported the isolation of infectious MV in BAL cells taken from infected macaques prior to the onset of viraemia at 3 d.p.i.. In addition, a large proportion of these cells are alveolar macrophages (CD11c+ MHC-II+) and lymphocytes which an epithelial-blind MV should infect with the same efficiency as wild-type MV. Taken together these data suggest that while epithelial cells are not involved in the initial stages of measles, infection could potentially initiate in areas of epithelium lining the respiratory tract which have been disrupted due to mechanical and/or viral infections (Fig. 1 A).

In contrast to the low level of MV-infected epithelial cells observed in the macaque model system, CDV, a morbillivirus which infects a wide-range of carnivore species, infects large numbers of epithelial cells in multiple tissues in infected ferrets (69). This suggests that CDV may have a higher affinity than MV for an unknown receptor expressed on epithelial cells or alternatively the longer in-vivo disease course of distemper compared to measles could result in increased cell-to-cell spread of virus within epithelial cell layers. The question of whether MV can utilise canine and bovine homologues of the unknown receptor, in a similar manner to the ability of the virus to use homologues of human SLAM as an entry receptor will be an interesting issue to resolve once the identity of this protein is established.

In the MV-infected human, endothelial cells of dermal capillaries and of small blood vessels throughout the body are claimed to show clear evidence of MV-infection (70). This phenomenon, if substantiated, could play a central role in pathogenesis leading to changes in the skin, conjunctivae, mucous membranes, and the brain, accompanied by vascular dilatation, increased vascular permeability, inflammatory cell infiltration, and infection of surrounding tissues (71–73). However, to our knowledge, double staining experiments (or in-situ hybridisation combined with staining) on MV-infected skin with MV antibodies and endothelial cell markers have not been published. In rare cases severe haemorrhagic infection with confluent haemorrhagic skin eruptions and intravascular coagulopathy, so called haemorrhagic or black measles occurs (74). In fatal cases of acute measles infection of capillary endothelium of lymph nodes and thymus has been described (75), though again without double staining of tissue for MV and host cellular proteins. Again in fatal measles widespread central endothelial infection has been described (76). In subacute sclerosing panencephalitis (SSPE), a small number of infected endothelial cells have been observed in a few cases (77–79).

To summarise, the evidence that MV can infect endothelial cells in vitro is overwhelming (50) and there is undoubted evidence for limited infection in natural measles. The mechanism by which the central nervous system (CNS) is infected either in acute measles or SSPE is unknown and the necessary receptor unidentified. One possible route of infection is through CNS endothelial cells, perhaps during the exanthema, when other endothelial cells are infected (Fig. 1 B) (79). Access into the brain by circulating inflammatory cells is also possible (73). In order to analyse whether MV is transported via transmigrating leukocytes across endothelial barriers, or whether virus spreads via infection of endothelial cells and basolateral release, we investigated the migratory behaviour of infected human primary T lymphocytes across polarised cell layers of human brain microvascular endothelial cells (HBMEC). We found that the capacity of lymphocytes to migrate through filter pores was only slightly affected by wild-type MV infection, whereas their capacity to migrate through endothelial barriers was drastically reduced (80). MV infection stimulated the expression and activation of the leukocyte integrin lymphocyte function associated antigen-1 (LFA-1) and very late activation antigen-4 (VLA-4) mediating a strong adherence to the surface of endothelial cells. Furthermore, the formation of engulfing membrane protrusions by endothelial cells, so called transmigratory cups, was induced, but transmigration was impaired. As a consequence of this close cell-cell contact, MV infection was transmitted from lymphocytes to the endothelium. MV envelope proteins were expressed on the apical and basolateral surface of infected polarised endothelial cells, and virus was released from both sides. Wild-type MV infection did not induce the formation of syncytia suggesting virus spread from cell-to-cell occurs via cellular processes and contacts. These data demonstrate in tissue culture that transendothelial migration of T cells (and similarly B cells and monocytes, S. Dittmar, personal communication) is strongly inhibited by MV infection, whereas virus can cross endothelial barriers by productive infection of the endothelium and subsequent bipolar virus release (80).
In vivo, this may mean that MV crosses the blood-brain barrier (BBB) by infection of microvascular endothelial cells, or that only a very limited number of infected lymphocytes may cross the BBB as reflected by the relatively rare number of cases of SSPE. Alternatively, uninfected leukocytes may transmigrate at certain sites and pave the way for further spread of uninfected and small numbers of infected lymphocytes into the underlying organs. Another hypothesis is that a secondary stimulus or immunosuppression may be necessary for a transient opening of the BBB. This „dual hit“ hypothesis was formulated and tested in a CD46-transgenic mouse model, in which lymphocytic choriomeningitis virus (LCMV clone 13) was used as an immunosuppressive agent (81). However, since studies in macaques cannot be used as model system for SSPE, to solve these questions for human infections, we must rely on histological analyses of post mortem obtained human autopsy specimens, which do not exactly reflect the situation months or years earlier, when MV possibly entered the CNS.

Conclusions and perspectives

In summary, accumulating data support the hypothesis that CD150 (SLAM) is the principal receptor required for the infection of initial CD150-positive target cells in the respiratory epithelium, for the infection of various cells of the immune system, and the manifestation of the profound immunosuppression. The further spread of MV across endothelial barriers is not completely understood, but infection of dermal capillary endothelial cells and microvascular endothelial cells in the brain has been observed. In late stages of measles, possible infection of epithelial cells lining the respiratory tract via the basolateral side, may suggest a role for this cell type in mediating the spread of virus from one individual to another. However, the histological detection of only limited numbers of such cells in vivo warrants further investigation into the precise mechanism(s) utilised by MV to cross epithelial cell barriers.

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Measles virus overcoming epithelial and endothelial barriers

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