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Modulation of immune functions by measles virus

Sibylle Schneider-Schaulies, Volker ter Meulen

Institute for Virology and Immunobiology, University of Wuerzburg, Versbacher Str. 7,
97078 Würzburg, Germany

Abstract. Measles virus remains among the most potent global pathogens killing more than 1 million children annually. A profound suppression of general immune functions occurs during and for weeks after the acute disease, which favors secondary infections. In contrast, virus-specific immune responses are efficiently generated, mediate viral control and clearance and confer a long-lasting immunity. Because they sense pathogen-associated molecular patterns, and subsequently initiate and shape adaptive immune responses, professional antigen-presenting cells (APC) such as dendritic cells are likely to play a key role in the induction and quality of the virus-specific immune response. Key features of immune suppression associated with measles virus, however, are compatible with interference with APC maturation and function and subsequent qualitative and quantitative alterations of T cell activation.

Introduction

Measles is considered one of the most important vaccine-preventable clinical entities worldwide. Implementation of the available live vaccine successfully eliminated indigenous transmission of measles virus (MV) in the Americas, and significantly reduced the number and magnitude of epidemics, morbidity and mortality in industrialized countries. In developing countries there was no such success and there the vast majority of the more than 40 million cases of measles occur, with more than 1 million fatal cases annually. Based on the general suppression of immune functions by MV, secondary infections mainly by microbial pathogens occur which are further aggravated by malnutrition. In infants of less than 1 year of age, maternal antibodies prevent successful vaccination, but not with wild-type MV infection.

Measles pathogenesis, immune activation and suppression

After primary infection of respiratory tract epithelial cells, MV is most likely acquired from the basolateral side of these cells by tissue-resident macrophages or

Correspondence to: S. Schneider-Schaulies

dendritic cells (DC) [82, 84, 86]. The virus then gains access to the local lymphatic tissues, where it replicates and spreads by a cell-associated viremia. MV-specific RNA and proteins can be detected in a minor proportion of lymphocytes and monocytes during and for a few days after the rash [36,120]. Little is known about the activation and the protective role of innate immune responses to MV. While conflicting data were reported on fluctuation of natural killer (NK) cell numbers following vaccination, expansion of these cells in natural measles evidently occurs [100, 146]. Interestingly, NK cell activity was found to be lower than in uninfected individuals [46]. Studies available on the induction of type I IFN during measles or after vaccination are inconclusive [30, 132, 138]. Activation of monocytes and/or DC apparently occurs as indicated by monocytosis and spontaneous release of IL-6 from peripheral blood mononuclear cells (PBMC) [12, 43]. The rash marks the onset of adaptive immune responses. Initially, virus-specific IgM and IgA antibody isotypes are produced, followed by IgG (mainly IgG1 and IgG4). IgE plasma levels also rise, but there is no evidence that the IgE contains MV-specific antibody. Infiltration of mononuclear cells into local areas of viral replication is seen and virus-specific T cells appear in the blood. Plasma levels of soluble T cell surface molecules CD4, CD8, IL-2 receptor and β_2-microglobulin are elevated, as are IL-2, IL-4 and IFN-γ. During measles, a switch from an initial Th1 to a long-lasting Th2 response occurs; an increase in IFN-γ during the rash is followed by an elevation of IL-4 plasma levels, which remains elevated for several weeks (for review see [41]). It has not been clearly resolved as yet whether generation of Th1 responses is less efficient after vaccination than after natural infection [40,124,146].

While MV-specific immunity is so efficiently generated, there is also a generalized suppression of immune responses during and for weeks after acute measles. A marked lymphopenia and a loss of delayed-type hypersensitivity reactions are characteristic findings. Considered as one of the major hallmarks of MV-induced immunosuppression, expansion of lymphocytes in response to polyclonal and antigen-specific stimulation ex vivo is severely impaired [123] for up to several weeks after acute measles, and also, albeit to a moderate extent, after vaccination [54]. A cytokine imbalance as seen by a predominant Th2 response and thus suppression of cellular immunity, was also suggested to contribute to impairments of cellular immunity [41]. Although MV was the first pathogen recognized to cause immunosuppression, it was only in the recent past that underlying mechanisms were partially unraveled and their relative importance for deregulation of lymphocyte numbers and function was addressed.

Complications of acute measles are frequent, and are common early after MV infection, when disturbances of immune function are apparent. They mainly affect the respiratory and intestinal tract (reviewed in [60]). In industrialized countries, about 10% of MV cases present with otitis, pneumonia, diarrhoea or encephalitis. CNS complications may occur early (acute measles encephalitis, thought to be a virus-induced autoimmune disease). Late CNS complications (subacute sclerosing panencephalitis, SSPE, or measles inclusion body encephalitis, MIBE) develop on the basis of a persistent MV infection in brain cells months to years after the acute infection. SSPE occurs in the face of a humoral hyperimmune reaction. Both diseases are invariably fatal and, on molecular level, restrictions of viral gene expression are characteristically seen (reviewed in [116]). In developing countries, MV infections are associated with high morbid-

ity and mortality rates. Pneumonia and diarrhoea are often complicated by secondary viral, bacterial and parasitic infections due to MV-induced immunosuppression.

The monotypic nature of MV has masked the existence of several cocirculating genotypes [108, 110]. Genetic characteristics of MV were defined by sequencing the genes coding for the hemagglutinin (H) and the nucleocapsid (N) protein, where the 450 nucleotides encoding the C-terminal region show up to 12% variability between individual strains. MV strains were assigned to eight clades (A–H), and within these more than 20 genotypes were designated, which reflects the genetic drift of MV strains [108, 110]. Multiple genotypes within a given clade are indicative of a high activity [109]. Molecular epidemiological studies have made significant contributions to measles control efforts as the source and transmission pathways of MV strains can be traced, as can the interruption of indigenous transmission in some areas. There is also no evidence that MV-wildtype strains differ in terms of pathogenicity or neurovirulence. Apparently, host rather than viral determinants are associated with MV pathogenicity such as immunocompetence. The basis for the attenuated phenotype of MV vaccine strains is unknown. Amongst other determinants, it is likely that sequence motifs within the glycoprotein genes common to the vaccine strains are important for attenuation since these proteins essentially determine viral cellular tropism and spread.

Measles virus

The viral core is a pleomorphic ribonucleoprotein particle (RNP), consisting of the nonsegmented RNA genome of negative polarity tightly encapsidated by N proteins and associated with the polymerase complex (Fig. 1A). The matrix (M) protein links the RNP to the lipid envelope from which the two viral glycoproteins, the fusion (F) and the H proteins, project. The M protein is also thought to interact with the cytoplasmic domains of the viral glycoproteins. In functional terms, this is revealed by the enhanced fusogenicity of recombinant MV lacking the M reading frame [28] and ability of the M protein to specify apical virus release and sorting of the viral glycoproteins in epithelial cells [86]. The H protein, a type II glycoprotein, mediates attachment of virions to cellular surface proteins, and also provides a helper function for membrane fusion [97,150]. Viral and membrane fusion requires the MV glycoprotein complex consisting of an H protein tetramer and an F protein trimer [75,106]. The latter protein, a type I glycoprotein, is synthesized as a precursor protein (F_0). Proteolytic activation by a cellular subtilisin-like protease, furin, into the disulfide bond-linked F_1/F_2 heterodimer induces a first conformational change [22, 64]. A second conformational change within the F_1/F_2 heterodimer occurs after receptor binding of the H protein, which leads to pairing of two amphipatic α-helical domains, thereby bringing the membranes to be fused in close proximity [15, 67] (exemplified for CD150 in Fig. 1B). Consequently, abolishment of F_0 protein activation by mutation of the cleavage site, or inclusion of peptides that interfere with the second conformational change strongly reduce or completely abolish infectivity [25, 68, 74]. Nonstructural proteins encoded by the MV genome include the C and the V proteins. Both are dispensable for viral replication in vitro [107, 114], but may play a role in viral pathogenesis in vivo [105, 140, 142].

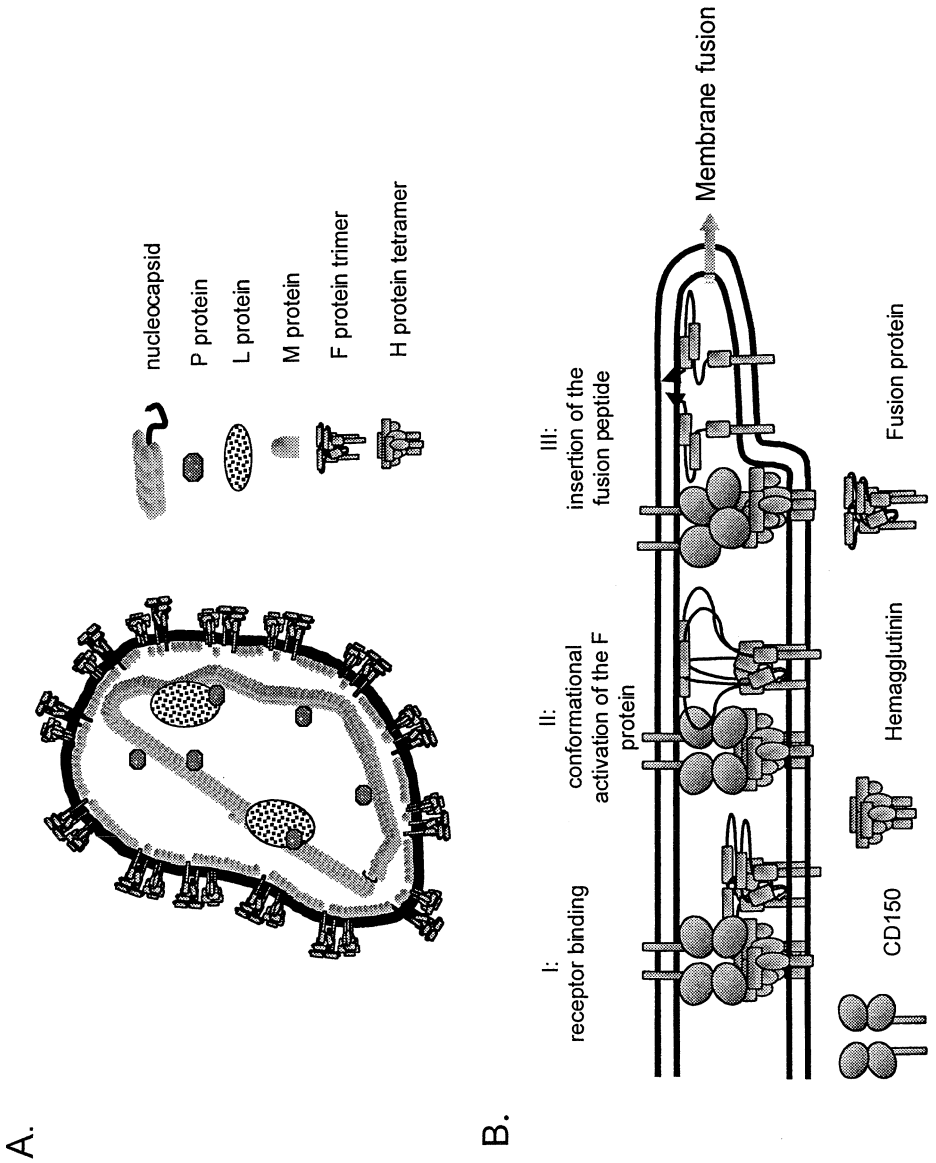


Fig. 1. A Schematic representation of the MV particle. **B** Model of MV induced membrane fusion; conformational changes within the F_1/F_2 heterodimeric protein. Interaction of the MV H protein with its cellular receptor CD150 triggers a conformational change within the F_1/F_2 heterodimer, which enables insertion of the hydrophobic fusion domain (located at the N terminus of the F_1 subunit) into the target cell membrane. A coiled coil structure is formed by intramolecular rearrangements within this subunit due to the interaction of the two α-helical domains within the F_1 subunit. Thereby, the membranes to be fused are brought in close proximity, which is a prerequisite for the mixing of the outer leaflets and subsequent fusion (*MV* measles virus)

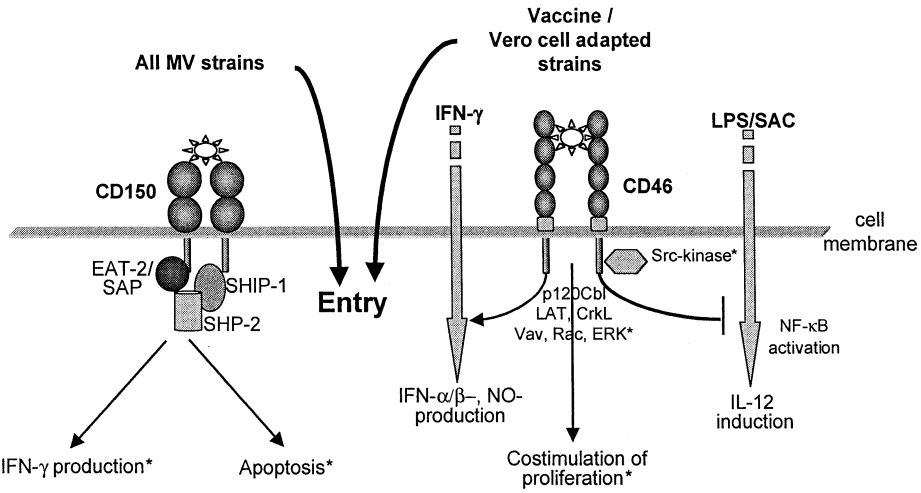


Fig. 2. Receptor interactions of MV and consequences on cellular signaling pathways. CD46 (for certain MV strains) and CD150 (for all MV strains) can support viral entry. Both molecules also have signaling properties. Ligation by specific antibodies triggers CD150 activation and subsequent signaling is dependent on the presence of adaptor molecules (SAP for T and NK cells and EAT-2 for antigen-presenting cells). CD150 activation is involved in recruitment and/or activity of phosphatases with protein (SHP-2) or dual specificity (SHIP-1), and in the induction of IFN-γ or cellular apoptosis. Src-family kinases mediate tyrosine phosphorylation of the cytoplasmic domain of CD46. In transgenic murine monocytes, CD46 ligation by MV led to enhancement of IFN-γ-primed release of IFN-α/β, and subsequent NO production. CD46 ligation can also suppress LPS or SAC stimulated release of IL-12 from monocytes. In T cells, CD3/CD46 coligation stimulated proliferation, and activation of signaling components. *Asterisks* indicate not documented as yet to occur by MV contact/infection

Receptor usage and consequences

CD46 (membrane cofactor protein, MCP) is ubiquitously expressed on human nucleated cells and was the first cellular receptor identified for MV [32, 89] (Fig. 2). Multiple isoforms of this protein generated by alternative splicing exist and are expressed in a tissue-specific manner. All CD46 isoforms support MV entry after transgenic expression in some, but not all rodent cell lines [77]. Transgenic expression of CD46 also generally does not confer susceptibility to MV infection by the respiratory route in rodents [21, 52, 96] although in one transgenic strain MV replication in peripheral tissues was reported [101]. Disruption of the type I IFN system was found to enhance susceptibility to MV infection in a CD46 transgenic mouse line [85]. The ability of MV H protein to interact with CD46 was assigned to some amino acids, with amino acid 481 being particularly important [18,70,91]. An extended binding site within CD46 spanning the two most membrane-distal short conserved domains (SCR1 and 2) interacts with MV H protein, while binding sites for the 'natural' ligands, the C3b/C4b complement components, map in SCR3 and 4 [27]. High-affinity binding to and usage of CD46 as receptor is, however, confined to attenuated vaccine strains and wild-type strains adapted to growth on Vero cells. Conflicting results were obtained with respect to the ability of clinical MV isolates to interact with CD46: while in one study two MV strains isolated and passed on PBMC were able to use CD46 as receptor [76], another study provides compelling evidence that MV of

throat swabs without any passage history do not interact with this molecule [102]. These and all other MV strains tested so far rather use CD150 as entry receptors (SLAM; signaling lymphocyte activation molecule), a member of the CD2 subset of the Ig superfamily [35, 53, 102, 137] (Fig. 2). Again, the most membrane distal part of CD150, the V domain, is essential for its function as MV receptor [103]. The expression pattern of CD150, which is found on activated T and B cells, memory cells and immature thymocytes, seems to be compatible with the well-recognized tropism of MV for cells of the lymphoid lineage. Strikingly, however this molecule is induced on DC only upon maturation and is not expressed by unstimulated monocytes [81, 98]. As suggested by recent findings, monocytes can be stimulated to express CD150 upon activation or infection with MV wild-type strains, and uptake of these strains into monocytes was found to be strictly dependent on CD150, but not CD46. It thus remains enigmatic how wild-type MV is acquired by primarily unstimulated monocytes which are amongst its major natural targets [36]. It also remains to be shown how these strains enter other CD150-negative cells, such as endothelial and epithelial cells during acute measles, or many other cells in complications such as dissemination in the immunoincompetent host or CNS infection. It is not known if, as seen with monocytes, other cell types can be induced to express CD150 upon inflammatory signals [81], or if additional receptors for MV exist. Although their role has not clearly been unraveled, molecules such as moesin [33,117] or substance P receptor [49] may play a role in MV entry.

Both CD46 and CD150 are down-regulated from the cell surface after contact with MV H protein and after infection [35,115,118]. For CD46, at least one consequence of MV-induced down-regulation is known. Corroborating the 'natural' function of CD46 to inactivate C3b/C4b deposits on the membrane, MV-induced CD46 down-regulation enhances sensitivity to lysis by activated complement of lymphocytes in vitro [119, 125]. Firm interaction with CD46 is a property of mainly Vero cell-adapted wild-type and vaccine MV, and thus this phenomenon may relate to attenuation by depletion of susceptible cells and thereby limiting viral spread. CD46 can also act as a signaling molecule, and downregulation of this molecule would thus be expected to have biological relevance. After CD46 ligation, tyrosine residues within its cytoplasmic domain 2 occurs in a src family-dependent manner [145]. Coligation of CD3/CD46 by antibodies enhances T cell proliferation and activation of signaling molecules such as by $p120^{CBL}$, LAT, CrkL, Vav, Rac and ERK, indicating that CD46 can act as a costimulatory molecule [8, 153] (Fig. 2). In CD46 transgenic murine macrophages, MV infection induced NO and type I IFN production in the presence of IFN-γ [59], and CD46 ligation by MV or CD46-specific antibodies, and also by C3b/C4b complement components, interfered with stimulated IL-12 synthesis in monocyte cultures (Fig. 2) [58]. Consequences of CD150 down-regulation remain speculative. Signaling events triggered after ligation of this molecule are complex and can lead to activation but also to cellular apoptosis [80,134]. Coupling of CD150 to intracellular signaling pathways requires adaptor proteins such as EAT-2 in antigen-presenting cells (APC) [83] or SAP in T and NK cells [69]. Interestingly, CD150 ligation in lymphocytes recruits SHIP-1, a dual specific phosphatase, and thereby regulates recruitment and function of intracellular signaling molecules [69]. IFN-γ production from $CD4^+$ T cells can be enhanced upon CD150 ligation [13], and thus it is tempting to speculate that down-regulation of this molecule may play a role in impaired Th1 responses as seen in measles [41]. On the other hand, ligation of CD150 by MV may also directly affect IFN-γ production from T cells.

Modulation of immune functions

In vivo studies of immunomodulation by MV were significantly hampered by the lack of suitable animal models permissive for respiratory tract infection. Amongst nonhuman primates, marmosets (*Saguinus mystax*) are most susceptible to experimental infection with MV wild-type strains and develop a usually fatal disease, which lacks, however, characteristic pathogenic features of human measles [5]. In cynomolgus, rhesus and squirrel monkeys (*Macaca fascicularis*, *Macaca mulatta* and *Saimiri sciureus*, respectively), the pathogenesis of experimental MV infection shares some features of the human infection such as the presence of infected PBMC, lymphopenia and immunosuppression [12, 79]. Intracerebral infection of mice and rats has contributed significantly to our understanding of pathomechanisms of MV-induced CNS infection [73]; however, these animals are generally nonpermissive for respiratory tract infection. In contrast, cotton rats (*Sigmodon hispidus*) can be intranasally infected with vaccine and wild-type MV [151]. In these animals, MV can be detected in PBMC at low frequency and infectious virus can be reisolated from lung tissue for up to 10 days [92]. Although not associated with clinical signs, infection of the respiratory tract epithelium and pneumonia with interstitial infiltrates is seen histologically [93].

Immunosuppression: features in vivo and ex vivo

Immunosuppression occurs shortly after appearance of the rash and may last weeks particularly in patients with complications of disease. A marked lymphopenia affects both the $CD4^+$ and $CD8^+$ T cell subpopulations without largely altering the $CD4^+/CD8^+$ T cell ratio [7, 99], although a reduced frequency of predominantly $CD8^+$ T cells was reported in the recovery phase [1, 2]. Mechanisms proposed to account for lymphopenia include virus-induced cell fusion or the induction of apoptosis. Ex vivo, an increased frequency of apoptotic PBMC during measles is not unequivocally documented, however, a considerable percentage of these cells underwent activation-induced cell death upon TCR triggering [1, 2]. Interestingly, a loss of peripheral T cells expressing high levels of LFA-1 was observed in the course of acute measles or after vaccination [87], and this could reflect aberrant lymphocyte trafficking by random homing to tissues. T cell lymphopenia is, however, not generally observed after vaccination, and is resolved within about 10 days after the rash during measles [100]. Thus, it cannot be causatively linked to impaired proliferative responses of mononuclear cells from patients in response to mitogenic, allogenic or recall antigen stimulation ex vivo, which is also seen, albeit less pronounced, after vaccination [54]. This unresponsiveness was partially resolved in vitro by neutralization of IL-4, but not unequivocally with IL-2 supplementation [23, 147]. Based on these observations, a cytokine imbalance strongly favoring a Th2-like response was suggested, which would result in a predominant humoral immune response and the generalized suppression of cellular immunity [45]. The recent observation that stimulated IL-12 release from monocytes of patients is apparent for prolonged periods would lend further support to this model [9]. In contrast, the ability of PBMC cultures isolated from vaccinees to produce IL-12 as well as IFN-γ has been directly demonstrated [40, 124]. In SCID mice grafted with human thymic material or human PBL, infection of thymic epithelial cells and thymocyte apoptosis as well as inhibi-

tion of antibody secretion, particularly after grafting of B cells from neonates, were observed following MV graft infection [11,139]. In cotton rats, inhibition of mitogen-induced B and T cell proliferative responses ex vivo was seen after intranasal infection [92]. Primary and secondary antigen-specific T cell responses are severely suppressed during MV infection in these animals, whereas B cells were only slightly affected [94]. Proliferation of T cells, but not generation of effector functions such as cytotoxicity or cytokine release were affected both after primary stimulation in vivo and secondary stimulation in vitro.

Mechanisms of immunosuppression in vitro

Although it is clear that virus-specific immune responses are successfully primed in measles and after vaccination, the exact mechanisms of immune activation by MV have rarely been addressed. It is very likely that uptake of MV by professional APC, particularly by DC, occurs with subsequent presentation of viral antigens to naive T cells. Most of the key features of MV-induced immunosuppression are, however, also compatible with viral interference with APC functions, and also with negative or apoptotic signaling to T cells.

MV interaction with professional APC

MV infection of monocytes is usually not productive even after activation of the cells. Cord blood monocytes support MV infection better than monocytes from adults, and immature myelomonocytic cells, but not those that have undergone in vitro maturation, support MV replication [50]. Deregulations of cytokine release from infected monocytes such as enhanced expression of IL-1β or reduced synthesis of TNF-α were seen in vitro and partially ex vivo [42, 72, 147]. In agreement with observations made in vivo [9], MV caused inhibition of stimulated IL-12 release in monocytes [58]. This occurred independently of viral replication and was brought about by ligation of CD46, and should thus be restricted to certain MV strains. High levels of MHC class II expression induced by MV infection of monocytes were found to enable efficient presentation of MV, but not of unrelated antigens [71], and this could contribute to the suppression of general, but not MV-specific, T cell activation. Since monocytes, however, cannot trigger activation of naive T cells, MV-dependent alterations of antigen presentation would mostly relate to recall antigens. For primary immune responses, MV interference with DC, would certainly be more relevant.

As ‘nature’s adjuvants’, DC play a major role in initiating and shaping the adaptive immune response to pathogens. With respect to their origin and cytokine pattern, two types of bone marrow-derived DC can be distinguished: DC1 are of myeloid origin and, upon activation, produce high levels of IL-12 and are considered as major stimulators of Th1 responses. In contrast, plasmacytoid DC (also referred to as DC2) produce IL-4, and only low levels of IL-12, but are major sources of Type I IFN. This is why they are also called natural interferon-producing cells (NIPC) in peripheral blood. In general, DC are located in most tissues and include Langerhans cells in the skin and mucous membranes, dermal DC, tissue-resident interstitial DC, veiled DC in blood and lymph, and interdigitating DC in the thymic medulla and secondary lymphoid tissues. In an immature state, DC are found in mucosal surfaces and in the

interstitial spaces in many peripheral tissues, where they capture and process antigen. Expression levels of MHC class II and costimulatory molecules are low to intermediate on these cells, and their capacity to stimulate allogeneic T cell proliferation in a mixed lymphocyte reaction in vitro is poor. Antigen uptake and signals provided in an inflammatory environment induce maturation and mobilization of DC. In secondary lymphatic tissues, they then appear as mature interdigitating DC, expressing MHC, costimulatory and adhesion molecules to high levels, in T cell-rich areas where they attract and aggregate T cells nonspecifically by producing a variety of chemokines [16,17]. DC stimulate T cell responses by displaying MHC-peptide complexes together with costimulatory molecules. Once activated, T cells induce terminal DC maturation by CD40 ligation, also accompanied by IL-12 production, particularly from the DC1 subtype [29]. In contrast to immature DC, mature DC are potent stimulators of allogenic T cell proliferation in vitro. If successfully infected, DC may, however, also have a role in viral propagation and dissemination. Moreover, targeting DC functions directly, viruses may exploit DC to induce an efficient suppression of immune functions as extensively studied for HIV [63]. Although likely, infection of DC in measles has not yet been documented. In experimentally infected rhesus macaques, syncytia formation, albeit to a very limited extent, was seen in lymph nodes; however, the cells recruited were not identified [79]. Evidence for infection of DC has been provided in genetically modified mice lacking the type I IFN receptor and transgenic for CD46 [84]. Due to receptor constraints, studies in these animals were restricted to CD46-adapted MV strains.

MV DC infection: impact on maturation. DC isolated from blood, Langerhans cells or DC generated in vitro from monocytes or $CD34^+$ precursor cells are susceptible to MV infection, and this causes extensive syncytium formation in both DC and mixed DC-T cell cultures [37, 47, 61, 62, 128, 130, 135]. In mixed cultures, both DC and T cell apoptosis also occurs [37], which may be induced by TRAIL from DC and/or expression of Fas ligand by T cells [129, 143] (Fig. 3A). Low production of infectious virus from DC can be enhanced by CD40 ligation [37, 130]. Interestingly, transmission of MV from DC to T cells seems to be restricted in DC-T cell cultures [37]. Not surprisingly, and similar to reports documented for LCMV [131], the MV glycoproteins are important for DC tropism. Thus, irrespective of their maturation stage, DC were more efficiently infected with a wild-type than with the Edmonston (ED) vaccine strain [98, 128]. Studies with recombinant MV, in which the authentic MV-derived glycoproteins were singly or doubly replaced by those of the WTF strain [57], revealed that expression of the ED H protein correlated with enhanced binding to lymphocytes and syncytia formation. Expression of WTF H, in contrast, enhanced viral tropism for DC by favoring entry, replication, and fusion [98]. The role of the MV receptors in this process is not entirely clear. While CD46 is expressed on DC [98, 128], CD150 may be considered as an activation marker on these cells inducible by LPS- or CD40 ligation [20, 65]. Up-regulation of CD150 was seen on LPS, but also MV wild-type-treated monocytes [81], and this might also occur in DC. This seems to imply that signaling via toll-like-receptors (TLR) is involved in up-regulation of CD150 at least with LPS [3]. Whether MV wild types act as agonists for TLR as recently confirmed for RSV [66] is currently unknown.

It is, however, clear that MV infection induces phenotypic maturation of immature DC, as expression of MHC class II and CD40 is augmented and costimulatory molecules such as CD80, CD86, CD83 and CD25 are induced [61, 128, 130]

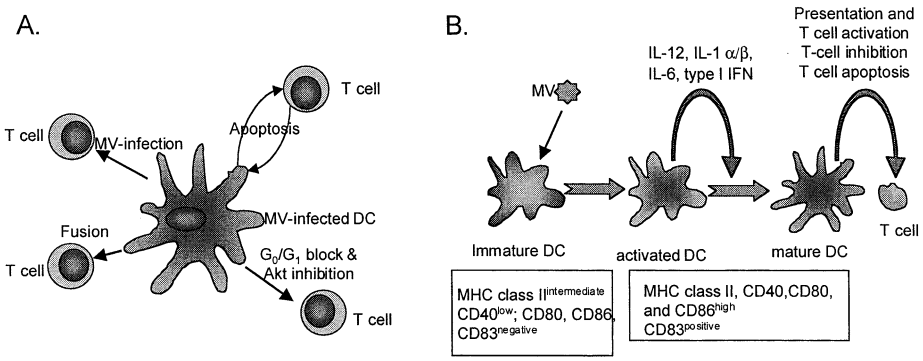


Fig. 3. MV – DC interactions: maturation and functional consequences. **A** T cell depletion can occur by DC/T cell fusion, transmission of infectious virus from DC, or TRAIL-mediated apoptosis. DC apoptosis may occur by activated T cells upon Fas ligation. Late in DC infection, the MV glycoproteins expressed to high levels on the surface efficiently inhibit allogenic- and mitogen-stimulated T cell proliferation by a contact-mediated mechanism. This F/H contact-mediated inhibition is associated with a disruption of Akt kinase activation, and is important in preventing S-phase entry of T cells. **B** Immature DC are activated and mature upon MV infection as indicated by up-regulation of MHC and costimulatory molecules and cytokine induction. Type I IFN can act in an autocrine and paracrine manner to trigger DC maturation. DC matured after DC interaction most likely home to the T cell-rich areas in secondary lymphatic tissues where they attract and interact with T cells. There, presentation of MV antigens, surface interactions and cytokines activate and shape T cell responses and this may effectively occur early after DC infection or after uptake (*DC* dendritic cell)

(Fig. 3B). Terminal maturation induced by CD40 ligand (CD40L), but not by LPS, was, however, found to be impaired in MV-infected DC by unknown mechanisms [130]. All MV strains tested to date were able to induce DC maturation. WTF, but not another wild-type strain, LYS-1, was more potent than the ED strain [47, 128, 135]. Infected DC release soluble mediators that promote maturation of immature DC, and using MxA protein as a marker, type I IFN was found to be one of them [61] (Fig. 3B). In spite of its ability to interfere with MV replication when overexpressed in monocytic and neural cells, MxA protein did not prevent viral spread in DC cultures [122, 126]. Thus, MxA may not accumulate to protective levels in DC or may not inhibit MV replication in these cells. Double-stranded (ds) RNA as accidentally formed during MV transcription and replication, is generally believed to induce type I IFN production. Recently, dsRNA was found to activate TLR3 and thereby type I IFN [6]. It is not known whether this pathway is also activated by MV. Surprisingly, however, induction of type I IFN in PBMC cultures was seen with MV vaccine strains, while wild-type strains even actively suppressed induction of this cytokine [90]. The underlying mechanisms remain to be established, and it is not known whether this is a general property of wild-type MV, and if so, whether differential induction of type I IFN also occurs in DC. Consistent with their activation, typical NF-κB-dependent transcripts for proinflammatory cytokines such as IL-12p40, IL-12p35, IL-1 α/β, IL-1RA and IL-6 were induced in DC by replication competent, but not UV-inactivated MV Halle strain [130]. NF-κB activation after MV infection is documented in a variety of cell types [31,48,51,55], and, although not formally proven, most likely occurs in DC as well. Conventional maturation stimuli of DC, such as LPS and SAC operate via TLR to trigger signaling cascades, which ultimately also converge in activation of NF-κB, and thereby promote the expression of co-

stimulatory molecules and cytokines such as IL-6, IL-1 α/β, TNF-α and IL-12 [4] (Fig. 2). It is possible that, in addition to viral replication, an MV glycoprotein might act as a TLR agonist as seen with the respiratory syncytial virus F protein [66].

MV interference with DC function. As outlined above, stimulated IL-12 release from PBMC of patients and monocyte cultures treated with MV was suppressed [9, 58]. In DC isolated from peripheral blood, IL-12 synthesis stimulated by bacterial cell wall components was unaffected by MV [128]. In contrast, in DC generated in vitro induction of this cytokine was suppressed by replication competent, and, to a moderate extent also by UV-inactivated MV [37, 130] (Fig. 2). Possibly, this obvious discrepancy is due to the different cell populations used, since peripheral blood DC mainly consist of the DC2 subtype, while those generated in vitro from monocytes have a DC1 phenotype and thus differ in their ability to produce IL-12. Of note, however, MV interference with IL-12p70 release was observed rather late after infection or treatment of MV with UV and may not occur to a significant extent earlier [37]. The mechanisms of IL-12 regulation in DC have not been resolved as yet, but may include negative signaling elicited by the surface interaction between CD46 and the H protein of CD46-adapted MV strains. Interestingly, inhibition of IL-12 production from DC was also inhibited in vivo in mice treated with the MV N protein indicating that other mechanisms also exist [78]. Targeting IL-12 release from monocytes or DC by MV by whatever mechanism should, however, have consequences for the quality of T cell responses, and could explain the inadequate Th1 response by measles suggested by some studies [43, 146]. Others confirmed, however, efficient induction of Th1 responses early in infection and following vaccination [40, 43, 44, 124]. Thus, it is possible that functional redundancies exist that compensate for a lack of IL-12 in priming of Th1 responses. In fact, in the human system, type I IFN can stimulate IFN-γ release from T cells directly, and independently of IL-12 [104], and type I IFN production from MV-infected DC is documented [61].

In spite of their mature phenotype, MV-infected DC are unable to stimulate allogenic, and even actively suppress in vitro-stimulated, T cell proliferation [37, 47, 61, 128, 130, 135] (Fig. 3A). The inhibitory activity is observed over a wide range of dilutions and is only partially neutralized by mature, uninfected DC [61, 128]. Although MV infection of T cells causes proliferative arrest (see below), this may not be significant here, since transmission of infectious MV to T cells by DC is inefficient [47]. Fusion and/or apoptosis seen in DC-T cell cultures may account to some extent for the reduction of T numbers, but not their unresponsiveness [129, 143, 144] (Fig. 3A). Inhibitory soluble mediators as released from MV-infected T or B cells [38, 136], were not detected in supernatants of MV-infected DC cultures [61]. More likely, the inhibition of T cell proliferation is largely brought about by the MV glycoprotein complex expressed on infected DC to high levels late in infection (Fig. 3A). This can be hypothesized because DC infected with a recombinant MV containing the vesicular stomatitis virus (VSV) G protein instead of MV glycoproteins retain a high allostimulatory activity and are not inhibitory for mitogen-driven T cell proliferation [61].

MV interaction with lymphocytes

Consequences of infection for viability and function. Mechanisms underlying the marked leukopenia during measles have not been clearly elucidated, however some

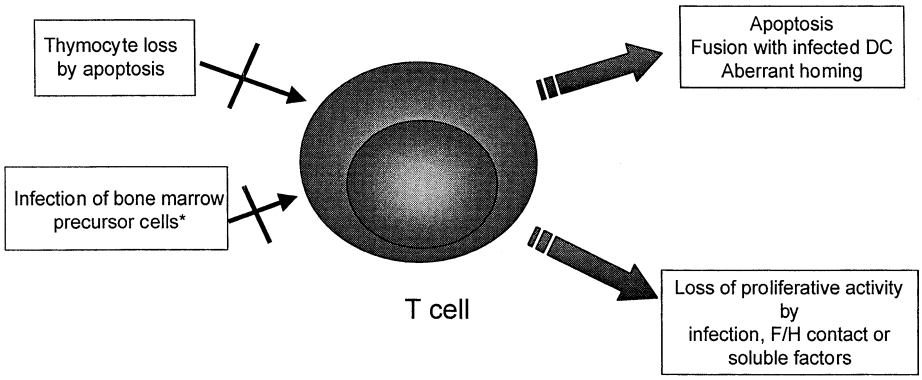


Fig. 4. Mechanisms contributing to loss or inhibition of T cells by MV. Potential MV infection of precursor cells may occur, but has thus far not been documented (*asterisk*). Thymocyte apoptosis by indirect mechanisms most likely results from MV infection of thymic epithelial cells. T cell loss may result from apoptosis, fusion with MV-infected DC or aberrant homing from peripheral blood. Expansion of peripheral blood T cells after polyclonal or antigen-specific stimulation is impaired. In a minority of cells, MV infection-induced growth arrest may occur. For the majority of these cells, which are not infected, indirect mechanisms such as the release of inhibitory cytokines from infected cells or surface contact-mediated negative signals, including those elicited by the F/H complex, are probably involved

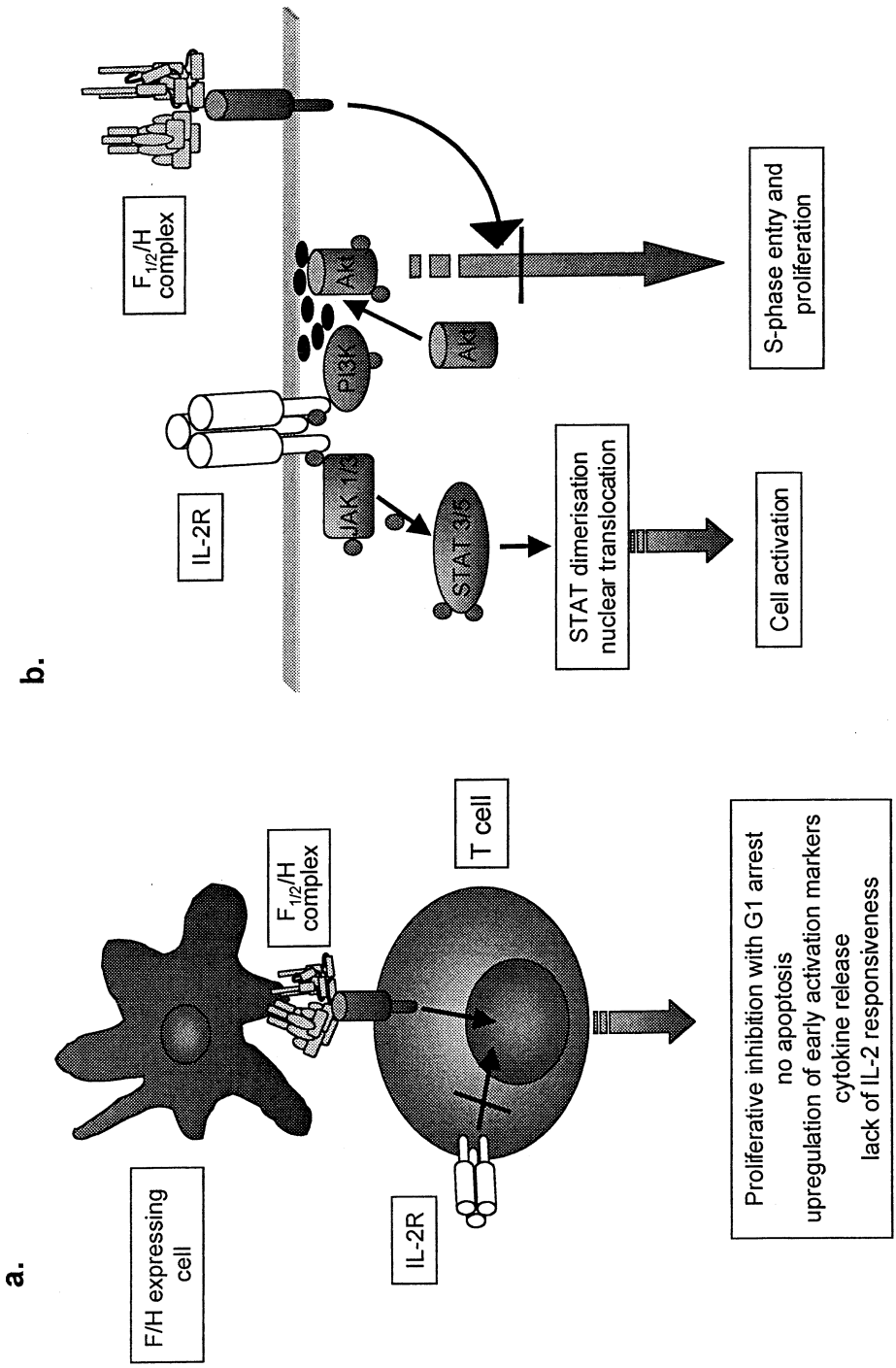
hypotheses have been put forward (Fig. 4). Infection of bone marrow precursor cells could interfere with the generation of lymphocytes, but this has not been experimentally addressed as yet. Depletion of uninfected lymphocytes by fusion with DC has already been referred to above. Lymphocyte apoptosis, as seen in MV-infected PBMC and T cell cultures stimulated with PMA/Ionomycin [56], could also contribute, but apparently requires ongoing viral replication since in mixed DC-T cell cultures it was only seen with live MV, but not with UV-inactivated MV [37] (Fig. 4). As the frequency of infected PBMC during acute infection is low, apoptosis induced by viral infection may not essentially account for lymphocyte depletion. Possibly, alterations of the thymic microenvironment and architecture impair efficient generation of immunocompetent T cells. In SCID mice engrafted with human thymus/liver implants MV infection induced thymocyte apoptosis [11]. MV infection was, however, confined to stromal thymic epithelial cells and monocytes, indicating that indirect mechanisms such as soluble mediators or surface contact-mediated signals are important (Fig. 4). MV infection in vitro caused a G0/G1 arrest in human thymic epithelial cells, and this was associated with terminal differentiation and, later on, apoptosis of these cells [141]. The possibility that aberrant homing by selective loss of LFA-1^{high}-expressing T cells may also contribute to leukopenia has already been referred to above [87]. In vitro, LFA-1 is up-regulated after MV infection of leukocytes [10]. ICAM-1, in turn, was up-regulated in endothelial cells by MV infection, and this correlated with the ability of the MV strain used to activate NF-κB [48]. The role of leukopenia for MV-induced immunosuppression in general is, however, unclear, since the duration of impairment of immune functions clearly exceeds the panlymphopenic state [99, 100].

In vitro MV infection of lymphocytes interferes with the generation of certain effector functions such as cytotoxic activity or B cell Ig synthesis [26, 39], which most likely results from MV-induced inhibition of lymphocyte proliferation. Although T

cell proliferation was markedly impaired after MV infection, mitogen-stimulated increase in cell volume, synthesis of early mRNA, up-regulation of early activation markers and IL-2 and IFN-γ production were unaffected by MV infection (for review see [121]). Similarly, in antigen-specific T cells or mitogen-stimulated PB lymphocytes, MV infection affected cellular proliferation, but not production of cytokines such as IL-2, IFN-γ, IL-6 or IL-10, whereas the production of IL-4 was reduced. In these cultures, down-regulation of the IL-2Rα subunit was observed [19]. In contrast, a general inhibition of cellular RNA synthesis was reported in MV-infected T cell cultures [88]. In this study, T cells accumulated in a G0-like state of the cell cycle characterized by reduced accumulation of cyclins D3 and E and high levels of $p27^{Kip1}$, an inhibitor of G1 cyclin-dependent kinases, maintained for prolonged periods. The block was, however, confined to a subpopulation of infected cells, whereas others, although infected, retained a normal proliferative response to mitogenic stimulation. It is not known whether the latter reflect a specific lymphocyte subpopulation that is refractory to MV infection-induced proliferative arrest, or whether the infection of these cells had not yet reached a level to abolish proliferative responses.

MV-induced inhibition of lymphocyte expansion: effector structures and mechanisms. Given the low number of infected lymphocytes in vivo, functional impairments and loss of proliferative activity are much more likely to be brought about by indirect mechanisms. These could include production of soluble inhibitory factors from infected cells, which would then act in an autocrine or paracrine manner. In fact, supernatants of MV infected B cells or T cells were found to inhibit antigen-specific proliferation of T cell lines or uninfected cells over a wide range of dilutions, but did not abolish the cytolytic activity of $CD4^+$ or $CD8^+$T cells [38, 136] (Fig. 4). The factors involved have not yet been identified, but a role of IL-10, TGF-β or type I IFN could be excluded. Type I IFN induced by MV infection of lymphocytes and monocytes is unlikely to play an essential role, because neutralizing anti-IFN antibodies did not affect mitogen unresponsiveness in infected T cell cultures [112, 136], and the abilities of MV strains to cause proliferative arrest and to induce type I IFN in vitro do not correlate [111].

The ability of MV glycoproteins expressed on the surface of infected cells to inhibit lymphocyte expansion was first seen in MV-infected T cell cultures where both uninfected and infected lymphocytes failed to proliferate in response to mitogenic stimulation, and this was abolished by anti-MV antibodies [112, 152]. Extending these observations, the MV glycoprotein complex on MV-infected, UV-irradiated cells and on viral particles was found to confer proliferative unresponsiveness to polyclonal stimulation in cells of the hematopoetic lineage. This has been directly revealed since fibroblasts transfected to express the F/H complex, but not those infected with a recombinant MV in which the MV glycoproteins were replaced by the VSV G protein, induced proliferative arrest of lymphocytes in vitro and in vivo [92, 113] (Fig. 5a). Inhibition, which required a short membrane contact to be induced, occurred independently of soluble mediators. Complex glycosylation and the fusogenic activity of the effector F/H complex were not required for inhibition, however, proteolytic processing of the F protein was necessary [148,149]. Inclusion of fusion inhibitory peptides, one of which prevents pairing of the two α-helical domains within the F_1 protein and, thereby, the conformational change essential for its fusogenic activity (see above), did not interfere with negative signaling by the glycoprotein complex. Thus, structural requirements for the fusogenic and inhibitory activity of


a.
F/H expressing cell
F1/2/H complex
T cell
IL-2R
Proliferative inhibition with G1 arrest
no apoptosis
upregulation of early activation markers
cytokine release
lack of IL-2 responsiveness
b.
F1/2/H complex
IL-2R
JAK 1/3
P13K
Akt
Akt
STAT 3/5
STAT dimerisation
nuclear translocation
Cell activation
S-phase entry and proliferation

this effector complex should differ. The receptor involved in this negative signal remains to be defined. In contrast to the observations made in CD46 transgenic mice [78], there was no requirement for CD46 or CD150 for the induction of T cell arrest. This is because the expression pattern of these molecules does not correlate with sensitivity to negative signaling, which was also unaffected by CD46- or CD150-specific antibodies [35, 95, 113]. F/H signaling does not induce lymphocyte apoptosis but rather a retarded passage of the G1/S-phase restriction point [95, 127] (Fig. 5a). At the molecular level, MV F/H-induced cell cycle retardation was associated with restrictions of the accumulation levels and the activity of proteins essentially controlling S-phase entry, such as the regulatory and catalytic subunits of cyclin-dependent kinases (CDK) and their inhibitor $p27^{Kip1}$ [34].

The expression levels of the IL-2Rβ and γ subunits were unaffected as was the stimulated induction of the IL2Rα-subunit, and IL-2 release by the effector complex and exogenously added IL-2 did not restore T cell proliferation [14,95]. Thus, it appears likely that IL-2R signaling pathways are targeted. In line with previous findings that T cell activation was not generally affected, the F/H complex did not interfere with IL-2-dependent activation of janus kinases (JAK) 1 and 3, which also efficiently activated signal transducers and activators of transcription (STAT) 3 and 5 [14]. In contrast, IL-2-dependent activation of the Akt kinase was disrupted after MV contact with T cells [14] (Fig. 5b). This kinase is activated by a variety of cytokines and growth factors which, after receptor interaction, catalyze phosphorylation of inositol-4,5- and inositol-5-phosphate into inositol-3,4,5- or inositol-3,5-phosphate, respectively, by phosphatidylinositol 3-kinase (PI3 K). This step is essential for membrane recruitment of pleckstrin homology-containing proteins such as the Akt kinase which, once membrane associated, is further activated by phosphorylation. This pathway plays a key role in transmitting survival signals in a variety of cell types and, particularly in T cells, in controlling CDK activity and S-phase entry [24].

In line with our observations that apoptosis is not induced by MV interaction, IL-2 stimulated functional inactivation by phosphorylation of the pro-apoptotic protein Bad occurred, which is mediated by the Akt kinase in some cell types. This finding suggested other kinases could be involved in Bad phosphorylation in T cells. Interference with Akt kinase activity by a pathogen provided a completely novel mode of modulation of T cell functions. Moreover, the importance of MV interference with Akt signaling for immunosuppression was revealed. First, IL-2 was largely unable to

◀ **Fig. 5.** MV F/H contact-mediated inhibition of T cell proliferation. **A** The F/H complex on the surface of infected cells, cells transfected to express these proteins, or viral particles, signals by interaction with an as yet unidentified receptor and abolishes lymphocyte expansion. In primary T cells, this contact-mediated signal does not affect mitogen-stimulated expression of early activation markers such as the IL-2R heterotrimer or release of cytokines including IL-2. Inhibition of T cell expansion by the MV F/H complex is not reverted by exogenous IL-2. **B** IL-2R activation triggers phosphorylation within its β- and γ-subunit cytoplasmic domains. Thereby, docking sites for JAK 1 and JAK 3 are created which, by autophosphorylation, then enable docking and phosphorylation of STAT3 and STAT5, which then dimerize and translocate to the nucleus. IL-2R signaling also activates PI3K, which catalyzes phosphorylation of inositol phosphates. These act as second messengers and mediate recruitment of pleckstrin-homology domain containing proteins such as the Akt kinase to the cell membrane. The Akt kinase is further activated by phosphorylation and subsequently regulates the activity of downstream targets. Signaling via this pathway is important for cell survival, but also, particularly in T cells, for S-phase entry. Negative signaling provided by the MV F/H complex interferes with IL-2-dependent activation of the PI3K/Akt pathway, but not with JAK/STAT activation (*PI3K* phosphatidylinositol 3-kinase)

activate the Akt kinase in lymph node cells isolated from experimentally infected cotton rats. Second, transgenic expression of a constitutively active Akt kinase (Myr-Akt) in Jurkat T cells or mitogen-stimulated mouse spleen cells strongly reduced their sensitivity to MV contact-mediated proliferative inhibition [14]. It also appears from the latter findings that MV contact-mediated interference with Akt kinase activation is not restricted to the IL-2R pathway, but is also seen after mitogenic stimulation of primary cells and IL-2-independent T cell growth. As mentioned above, activation of the Akt kinase is tightly regulated by upstream activation of PI3K, which could possibly be already targeted by MV signaling. Alternatively, the activity of lipid phosphatases such as PTEN and SHIP-1 and, thereby, the turnover of phospholipids is enhanced. Of note, SHIP-1 membrane recruitment and activity can be regulated by CD150 ligation [133]. Since all MV strains are able to bind to and use this receptor, it is tempting to speculate that activation of SHIP-1 via this pathway could contribute to Akt inactivation. On the other hand, immunosuppression as indicated by proliferative unresponsiveness after MV contact was found to occur independently of the presence of this molecule, and CD150 ligation by monoclonal antibodies was linked to T cell stimulation rather than inhibition [13].

Conclusions and future directions

Immunomodulation by MV is highly complex and involves both immune stimulating and inhibitory mechanisms. Although we are beginning to understand some levels of interactions, there are important questions concerning MV immunomodulation which are still unanswered. Given the central role of DC in the induction of the MV-specific immune response and possibly also in immunosuppression, MV interactions with these cells in vivo need to be addressed. For immune activation, MV might be taken up by endocytosis and processed for antigen presentation. MV infection might also induce cellular apoptosis.

By uptake of apoptotic bodies, uninfected DC could efficiently stimulate T cell responses, a phenomenon known as cross-priming. For migration to secondary lymphatic tissues and subsequent T cell activation stimulation, DC need to be activated and mature. The mechanisms by which MV accomplishes this are as yet unclear. It is also presently unknown whether the expression pattern of chemokine receptors and chemokines in these cells, of crucial importance for their migration and T cell recruitment, is regulated, perhaps differentially, by MV. It is, however, also quite possible that in early MV infection, during which low levels of MV glycoproteins are expressed on these cells, immune responses might be efficiently primed and shaped. In support of this hypothesis, the allostimulatory activity of MV-infected DC cultures *in vitro* is retained early after infection [61]. When the expression levels of the viral glycoproteins exceed a certain threshold, DC or DC /T cell fusion, DC or T cell apoptosis, and active T cell inhibition by DC could occur. Surprisingly, the impact of DC infection on the quality of T cell activation has not yet been addressed. Suppression of Th1 responses cannot necessarily be expected. Although it now seems clear that, at least late in DC infection stimulated production of IL-12 is reduced, it is quite possible that type I IFN, which is produced early in DC infection, may efficiently induce IFN-γ production by T cells. In view of their differential ability to produce IL-12 or type I IFN, it is tempting to speculate that MV in the DC1 or DC2 subtype might have different impacts on their cytokine profile and quality of the stimulated T cell responses.

Importantly, however, as with many other viruses for which interaction with DC was studied mostly in vitro, infection of DC by MV in vivo needs to be documented. For obvious reasons this can only be done in animal models. Rhesus macaques and cotton rats can be infected via the respiratory tract, and both animals are permissive for infection with wild-type and vaccine MV strains. As a small animal model and with inbred strains available, cotton rats appear particularly attractive for these studies, and once the reagents are available, will provide conclusive insights into the tropism of MV strains for DC, the type of DC infected, and their trafficking to lymphatic compartments.

Note added in proof

While this manuscript was under review, we established that the H protein at lymphotropic wild-type MV strains, but not vaccine strains, acts as an agonist for TLR2 on monocytes. This results in cytokine production, but also in the induction of CD150 surface expression on these cells (Bieback K. et al., J. Virol., in press)

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