Review

The Role of Sphingomyelin Breakdown in Measles Virus Immunomodulation

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Key Words

Measles virus • Immunosuppression • T cell silencing • Dendritic cell • Sphingomyelinase

Abstract

Measles virus (MV) efficiently causes generalized immunosuppression which accounts to a major extent for cases of measles-associated severe morbidity and mortality. MV infections alter many functions of antigen presenting cells (APC) (dendritic cells (DCs)) and lymphocytes, yet many molecular targets of the virus remain poorly defined. Cellular interactions and effector functions of DCs and lymphocytes are regulated by surface receptors. Associating with other proteins involved in cell signaling, receptors form part of receptosomes that respond to and transmit external signals through dynamic interactions with the cytoskeleton. Alterations in the composition and metabolism of membrane sphingolipids have a substantial impact on both processes. In this review we focus on the regulation of sphingomyelinase activity and ceramide release in cells exposed to MV and discuss the immunosuppressive role of sphingomyelin breakdown induced by MV.

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Introduction

As established more than a century ago, MV efficiently suppresses especially the adaptive immune response and this almost entirely accounts for the high rates of morbidity/mortality associated with acute measles [1]. Typically, patients succumb to secondary infections, which may follow a fatal course, and chronic or latent infections may exacerbate due to the loss of mainly T cell dependent immune control. Quantitative and qualitative alterations of peripheral blood lymphocytes typically occur during or for weeks after the acute infection. These include lymphopenia, an early switch from a Th1 towards a Th2 dominated response and a general inability of peripheral blood lymphocytes to expand

in response to polyclonal or antigen-specific stimulation *ex vivo* [1, 2]. The latter two characteristics mark MV as a pathogen specifically targeting cellular immunity for efficient generalised immunosuppression.

Interaction with its plasma membrane surface receptors is of major importance for measles pathogenesis also including immunosuppression [3, 4]. Though all of them are signaling active molecules, only three receptors confer viral uptake into target cells: CD46 (used by attenuated strains *in vitro* [5]), nectin-4 (expressed on the basolateral side of epithelial cells, and important for viral shedding from infected hosts [6, 7]), and CD150 (also referred to as SLAMF1 [8]). *In vivo*, MV uptake and hematopoietic spread clearly segregate with CD150 expression patterns [9, 10]. While MV interaction with pattern recognition receptors such as DC-SIGN and TLR2 was linked to enhancement of viral uptake into or maturation of APCs [11, 12], respectively, that with others such as FcγRII or an as yet undefined receptor (see below) was implicated in modulating lymphocyte viability and activity in immunosuppression [13, 14]. Within the following paragraphs we will highlight the role of stimulated sphingomyelin breakdown at the plasma membrane in two important aspects of MV induced immunomodulation: DC entry and T cell silencing.

MV-DC interactions: sphingolipid breakdown mediated enhancement of infection

The importance of the MV entry receptor, CD150, for major aspects of MV pathogenesis received direct support from studies performed in experimentally infected rhesus macaques. CD150 expression is confined to hematopoietic cells, and predominantly activated follicular B and memory T lymphocytes are infected in secondary lymphatic tissues and, at low frequency, in PBMCs [10, 15]. Lending support to the hypothesis that APCs in the lower respiratory tract are early, if not prime target cells for viral infection, MV infected CD150+ alveolar macrophages and CD11c+ MHC class-II+ myeloid DCs were detected in peripheral tissues early following infection [10, 16]. This suggests that MV initially interacts with immature DCs and exploits them as Trojan horses for transport into T cell rich areas in secondary lymphatic tissues where transmission to lymphocytes occurs [13, 14, 17, 18]. Unlike HIV, for which a similar scenario is postulated, MV efficiently infects DCs and this occurs in a CD150-dependent manner enhanced by DC-SIGN [12, 19]. DC-SIGN, in conjunction with TLR2, may also promote phenotypic and functional DC maturation [11, 12] which resembles that induced by other viruses in terms of MHC- and costimulatory molecule upregulation and cytokine release, though CCR7 upregulation and CD40 signaling may be compromised [20, 21]. To what extent these virus-induced alterations translate into the inability of MV-infected DCs to stimulate T cell proliferation is unknown [13, 14]. There is, however, evidence that the majority of conjugates formed between T cells and infected DCs is unstable and does not sustain Ca^{2+} mobilization in T cells [22]. It appears that in addition to inadequate expression and recruitment of repulsion receptors and their ligands inhibitory signals provided by the viral glycoprotein on the DC surface to conjugating T cells are essentially involved in this process [23, 24], thereby rendering MV uptake for subsequent replication highly important in DC-mediated T cell silencing.

Surface expression of the MV uptake receptor CD150 is low on myeloid DCs *in vitro* and *in vivo* [19, 25], and membrane compartmentalization or clustering of this receptor has not yet been addressed on these cells. Lateral segregation followed by receptor concentration also including those for pathogens was, however, described following acid sphingomyelinase (ASM) activation, subsequent ceramide release and condensation of ceramide microdomains into extended ceramide enriched platforms [26, 27]. DC-SIGN is the major MV attachment receptor on DCs [19, 28]. Its signaling involves activation of c-Raf which, in general, can be subject to ceramide mediated regulation [29, 30], and therefore, DC-SIGN appeared to be a likely candidate in promoting sphingomyelinase activation leading to MV receptor clustering. Indeed, DC-SIGN ligation by MV, but also specific antibodies after crosslink or mannan efficiently caused transient ASM activation and surface display of ASM and

ceramides, and this was sensitive to EGTA, amitriptyline and DC-SIGN blocking antibodies [25]. Pharmacological or siRNA-mediated genetic ablation of ASM did not affect MV binding to DCs, however, severely impaired uptake as detected by accumulation of viral proteins after 16 hrs. This was not observed in cell lines where the uptake receptor was abundantly expressed corroborating our hypothesis that DC-SIGN mediated ASM activation regulated entry receptor availability. While lateral co-segregation of abundantly expressed CD150 and transgenic DC-SIGN was observed after mannan exposure in Raji B cells, DC-SIGN ligation caused a transient increase in CD150 surface display within minutes in DCs indicating that in these cells, ASM activation caused vertical rather than lateral CD150 recruitment [25]. It appeared that CD150 was co-transported with ASM from a common intracellular storage compartment.

There are several implications of this study that extend beyond the MV system: DC-SIGN ligation is not unique to MV. Receptor sorting upon DC-SIGN mediated ASM activation and formation of ceramide-enriched platforms on DCs might well positively, but also negatively regulate pathogen uptake. Thus, sphingomyelinase exposure caused segregation CD4 from CXCR4 and thus inhibition of fusion-mediated HIV-1 uptake, and rather fostered viral endocytosis followed by lysosomal targeting [31]. Whether this would also occur in response to ASM activation has not been tested, nor has it been assessed in viral systems whether ASM activity supports membrane fusion as revealed for phago-lysosomal fusion in macrophages infected with *L. monocytogenes* [32, 33]. Thus, while consequences of DC-SIGN dependent ASM activation in pathogen uptake are not MV-specific, CD150 recruitment seemingly is. Because CD150 can, however, serve as pathogen recognition receptor routing gram-negative bacteria into an endolysosomal degradative compartment in murine macrophages [34], transient availability of this receptor could be important in co-infection settings. Apart from enhancing MV uptake, ASM activation was also found to be required for DC-SIGN proximal and downstream signaling [25]. While a role of ASM in membrane proximal c-Raf and ERK activation was not surprising, inhibition of NF- κ B upon TLR4 co-ligation was unexpected, because enhancement of LPS-driven NF- κ B activation upon DC-SIGN co-ligation was described to occur in DCs earlier [30]. While reasons for these discrepant observations have not been unraveled as yet, ASM activation in response to DC-SIGN ligation appears to regulate gene expression in DCs especially with regard to cytokine release, which, again, would be DC-SIGN, but not MV-specific.

MV-interaction with T cells: actin cytoskeletal paralysis and beyond

Several scenarios relating to MV T cell interference were described. These include 1) loss of viability occurring due to infection and/or infection-independent apoptosis. The latter can be caused by ligation of the Fc γ RII [35], while infection related loss may result from viral cytolysis, formation of multinucleated giant cells or destruction by virus-specific effector T cells in secondary lymphatic tissues [15]. Because this has been particularly revealed for the memory T cell population, both lymphopenia but also loss of recall responses during measles were attributed to this mechanism [15]. 2) A general cytokine imbalance was described *in vitro* and *in vivo* in that an initial Th1 rapidly shifts towards a Th2 response thereby disfavoring cellular immunity [1]. 3) Thirdly, expansion of T cells isolated from patients in response to polyclonal or antigen-specific activation is strongly impaired. The latter phenomenon has been mimicked by exposure of primary or transformed T cells to the MV glycoprotein complex provided by infected cells, cells transfected to express this effector complex or viral particles. T cell inhibition in this system does not involve infection or cell death, but rather a profound inability to pass beyond the G₁ phase [3].

It was for several reasons that regulation of phospholipid dynamics was likely of importance in MV T cell silencing. Binding of purified MV causes condensation of detergent resistant membrane microdomains on resting T cells and abolishes signaling initiated from the IL-2R and TCR at a membrane proximal level *in vitro* and *ex vivo* [36, 37]. MV targets activation of the PI3K, and consequently, generation of membrane phosphatidyl-inositol-

3,4,5-phosphate (PIP₃) and recruitment of PH domain containing proteins (including Akt kinase and the guanosin exchange factor Vav) [37]. Akt inhibition is crucial to MV T cell silencing, because this is efficiently abrogated upon expression of a constitutively membrane associated, active Akt kinase [36]. As they are PI3K downstream effectors, MV impaired nuclear accumulation of certain splice accessory factors thereby giving rise to production of protein isoforms translated from alternatively spliced mRNAs. These included a constitutively membrane associated active lipid phosphatase (SIP110) which on overexpression interfered with T cell expansion, most likely by constitutively depleting PIP₃ at the cell membrane [38].

As a second major target of MV T cell silencing, integrity and dynamics of cortical actin proved to be effectively disturbed (referred to as T cell paralysis) which is characterized by a failure to 1) adhere to and morphologically polarise on fibronectin (FN), 2) to recruit Vav and activate small GTPases Rac1 and Cdc42 and 3) to phosphorylate ezrin/moesin proteins and cofilin, which when dephosphorylated, severs barbed ends of actin filaments, upon TCR ligation [39]. Phenotypically, MV exposure causes a rapid collapse of actin based protrusions in T cells which, on stimulation, are unable to spread, form lamellopodia, and correctly organize monocentric immune synapses (ISs) with mature DCs [39]. Moreover, ISs formed between infected DCs and T cells were mostly unstable and did not support sustained Ca²⁺-fluxing thereby explaining why infected DCs do not promote T cell expansion [22, 24, 40]. Because both membrane phospholipid metabolism and actin dynamics are essential components of IS function [41, 42], it is not surprising that a virus interfering at these levels is highly efficient at abrogating T cell activation.

Several lines of evidence suggested that sphingomyelinase activation and ceramide release might contribute to MV-mediated T cell silencing mainly including their interference with Akt activation, cytoskeletal remodeling and ERM protein activity (though not revealed in T cells) [43-46] as well as their ability to hamper TCR-stimulated CRAC channel activation and subsequent Ca²⁺-influx [47, 48]. Moreover, inhibition of T cell proliferation was reported after NSM activation upon cholera toxin binding to lipid rafts or exogenous elevation of ceramides [49, 50].

Indeed, MV causes surface accumulation of ceramides due to activation of both NSM and ASM (the latter being displayed at the surface), with NSM most likely accounting for ASM activation. This was related to the MV gp interaction since a recombinant MV, expressing VSV G protein instead (MGV) failed to activate sphingomyelinases [3, 51]. MV-induced sphingomyelinase activation appeared not to account for loss of stimulated T cell proliferation, but rather for that of membrane protrusions and efficiently interfered with FN- or α CD3/CD28-stimulated T cell polarisation, surface receptor redistribution, ezrin/moesin phosphorylation, and directional cell motility. Bacterial sphingomyelinase or biologically active C₁₆-ceramide caused similar effects when applied instead of MV, and thus, our observations apparently reflect a general, as yet unnoted ceramide dependent block in T cell cytoskeletal dynamics and thereby, activation [51]. Interestingly, ASM deficient T cells revealed an aberrant morphology as well in that they developed prominent ruffles instead of microvillar structures and were unable to polarise their cortical actin on fibronectin (Avota and Schneider-Schaulies, unpublished). These observations indicate that temporally and spatially controlled sphingomyelinase activation may be of crucial importance in cytoskeletal dynamics in T cells thereby controlling essential functions such as adherence, migration, and interaction with APCs.

Outlook

Formation of unstable ISs with MV-infected DCs which do not support sustained Ca²⁺ mobilization in T cells strongly suggests that MV targets their architecture and function for T cell silencing. Because formation of functional ISs is critical to T cell activation, not surprisingly pathogens other than MV also target this interaction for intervention. This has been documented for DCs infected with respiratory syncytial virus or ISs involving HIV-infected T cells [52-55].

How sphingomyelin breakdown/ceramide release might regulate of IS function and what would be the targets is certainly a highly interesting subject to address. Little is known about the role of sphingomyelinase activity in DC maturation and function as yet nor has it been directly assessed whether association of surface receptors important in DC function (such as for instance CD80, CD86 or CD40) with microdomains is subject to regulation in response to sphingomyelinase activation. If, as revealed for other cell types, MV gps would be located in lipid rafts in DCs as well, they might redistribute upon sphingomyelin breakdown thereby possibly affecting interaction with receptors on T cells required for transmission of activatory signals. Much less studied as for T cells, actin remodeling and protrusion of actin based extensions are also critical for the ability of DCs to promote T cell activation [56], and it would be interesting to see whether this would be positively or negatively affected upon sphingomyelinase activation. As evident from several studies, sphingomyelinase activation/ceramide release interferes with T cell activation, for instance by affecting actin cytoskeletal remodeling and sustained Ca^{2+} mobilization [47, 48, 51], however, targets remain as yet obscure. In line with their inhibitory function, ceramide species are virtually absent from the lipid environment of activated TCR clusters [57]. It will thus be highly interesting to directly reveal as to whether infectious agents (or compounds thereof) interfering with IS function would cause either of these effects, and if so, define microdomains targeted within the IS. This, however, will only become feasible once the experimental tools will become available to address this issue.

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We disclose any sponsorship relating to our research and conflicts of interest.

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