MINIREVIEW

Measles Virus Receptor SLAM (CD150)

Yusuke Yanagi, Nobuyuki Ono, Hironobu Tatsuo, Koji Hashimoto, and Hiroko Minagawa

Department of Virology, Faculty of Medicine, Kyushu University, Fukuoka 812-8582, Japan

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INTRODUCTION

Measles virus (MV), a member of the Morbillivirus genus in the Paramyxoviridae family, is an enveloped virus with a nonsegmented negative-strand RNA genome and infects humans and nonhuman primates (Griffin, 2001). Despite the availability of vaccines, MV remains a major cause of childhood mortality, claiming roughly one million lives a year worldwide. The transient immunosuppression that accompanies and follows measles renders the patients susceptible to secondary infections accounting for most of measles-related complications and deaths. MV also causes postinfectious encephalitis, and in rare instances, subacute sclerosing panencephalitis, a persistent infection in the central nervous system. This review is concerned with the identification of a new MV receptor and its implication for understanding the pathology and pathogenesis of MV infection.

MV was first isolated in primary human kidney cells inoculated with the blood and throat washings of a child with measles (Enders and Peebles, 1954). This first isolate, the Edmonston strain, was subsequently adapted to chicken embryo fibroblasts and became the progenitor for currently used attenuated vaccines. The Edmonston strain also grows well in continuous cell lines and has become the most extensively studied MV strain in laboratories. Vero cells, an African green monkey kidney cell line, had been commonly used for MV isolation until a decade ago, but several blind passages were usually required before virus propagation and development of cytopathic effect (CPE). Kobune et al. (1990) reported that an Epstein–Barr virus (EBV)-transformed marmoset B cell line B95-8 and its adherent subline B95a were highly sensitive to MV. Furthermore, they showed that MV strains isolated in B95a cells, but not Vero cell-isolated strains, retained pathogenicity for monkeys (Kobune et al., 1990, 1996). Thus, the use of B95a is currently recommended for MV isolation from clinical specimens (World Health Organization, 2001). Subsequently, other human B cell lines have been also successfully employed for MV isolation (Schneider-Schaulies et al., 1995b; Lecouturier et al., 1996).

In 1993, human CD46 (also known as membrane cofactor protein) was identified as a cellular receptor for the Edmonston and Halle strains of MV (Dorig et al., 1993; Nanichte et al., 1993a). CD46 is a complement regulatory molecule and is expressed on all nucleated cells in humans. The Edmonston and Vero cell isolated strains are capable of infecting any CD46/ H11001 primate cell lines. On the other hand, B cell isolated strains grow in a restricted number of B and T cell lines and phytohemagglutinin-stimulated peripheral blood mononuclear cells (PBMC), but not in other CD46/ cell lines (Schneider-Schaulies et al., 1995b; Lecouturier et al., 1996; Hsu et al., 1998; Tanaka et al., 1998; Tatsuo et al., 2000a). Furthermore, the Edmonston strain causes hemadsorption with monkey red blood cells (which express CD46, unlike human red blood cells) and CD46 downregulation from the surface of the infected cells, whereas B cell isolated strains do not (Saito et al., 1992; Nanichte et al., 1993b; Schneider-Schaulies et al., 1995a; Lecouturier et al., 1996).

MV has two envelope glycoproteins, the hemagglutinin (H) and fusion (F) protein, mediating receptor binding and membrane fusion, respectively (Griffin, 2001). Several studies provided evidence that the H protein of the Edmonston strain, but not of B cell isolated strains, interacts with CD46 (Lecouturier et al., 1996; Hsu et al., 1998; Tanaka et al., 1998), suggesting the presence of another receptor for B cell isolated strains. Others, however, argued that B cell isolated strains can enter cells using CD46, but fail to replicate in nonlymphoid cells (Schneider-Schaulies et al., 1995b; Manchester et al., 2000).

1 To whom correspondence and reprint requests should be addressed. Fax: +81-92-642-6140. E-mail: yyanagi@virology.med.kyushu-u.ac.jp.
IDENTIFICATION OF A NEW CELLULAR RECEPTOR FOR MV

To determine the mechanism underlying the different cell tropism of the Edmonston and B cell isolated strains, we utilized the pseudotype system based on the recombinant vesicular stomatitis virus (VSV) containing the green fluorescent protein as a reporter (Takada et al., 1997). Since this recombinant VSV lacks the gene encoding the G envelope protein, it can only enter cells using envelope proteins provided \textit{in trans}. However subsequent intracellular steps progress as part of the VSV replication cycle. Our study using this system demonstrated that the difference in cell tropism between the MV strains was largely determined by viral entry (Tatsuo et al., 2000b). A single clone was obtained that could make the transfected 293T cells highly susceptible to the pseudotype. This marmoset cDNA clone had a high level of similarity to the human signaling lymphocyte activation molecule (SLAM; also known as CD150) gene, suggesting that human SLAM, a membrane glycoprotein involved in lymphocyte activation (Cocks et al., 1995), is a cellular receptor for this and other B cell isolated MV strains that cannot use CD46 as a receptor.

Further experiments showed that expression of human SLAM conferred on nonsusceptible human kidney cell line 293T was transfected with a cDNA library of B95a cells and then screened with the VSV pseudotype bearing the H protein of a B cell isolated MV strain and F protein of the Edmonston strain (Tatsuo et al., 2000b). A single clone was obtained that could make the transfected 293T cells highly susceptible to the pseudotype. This marmoset cDNA clone had a high level of similarity to the human signaling lymphocyte activation molecule (SLAM; also known as CD150) gene, suggesting that human SLAM, a membrane glycoprotein involved in lymphocyte activation (Cocks et al., 1995), is a cellular receptor for this and other B cell isolated MV strains that cannot use CD46 as a receptor.

Since rodent cells such as Chinese hamster ovary (CHO) cells that do not express CD46 are able to support MV growth after expression of human SLAM, CD46 is not required for SLAM to act as a receptor. Importantly, the Edmonston strain can use both SLAM and CD46 as receptors. MV strains isolated in PBMC have been reported to use CD46 as a receptor (Manchester et al., 2000), but they are also able to use SLAM as a receptor (Tatsuo et al., 2000b). Since these PBMC isolates produce CPE in SLAM-expressing CHO cells, but not in CD46-expressing CHO cells, SLAM may serve better as a receptor for these strains than CD46. Expression of SLAM on various cell lines correlates with their susceptibility to B cell isolated MV strains. B95a cells express a high level of SLAM, which may explain why they have been useful in isolating MV from clinical specimens. Shortly afterward, two other groups confirmed all our findings by using different approaches (Erlenhoefer et al., 2001; Hsu et al., 2001).

THE PRINCIPAL MV RECEPTOR IN THE BODY

Viruses isolated in cultured cells may not be representative of those \textit{in vivo} because of \textit{in vitro} selection and/or adaptation. To ascertain \textit{in vivo} relevance of SLAM as an MV receptor, we inoculated human SLAM-expressing CHO cells with throat swabs from measles patients. The cells developed CPE at 36 h after inoculation with throat swabs, and syncytia formed were strongly stained with anti-MV antibody (Tatsuo et al., 2000b), indicating that SLAM can act as a receptor for MV in the body.

No MV strains have been found that cannot use SLAM as a receptor, whereas only the Edmonston and some other strains can use, besides SLAM, CD46 as a receptor. Furthermore, MV isolates are more rapidly and efficiently obtained from clinical specimens, in B95a cells than in Vero cells (Kobune et al., 1990; Griffin, 2001). These observations suggest that the majority of MV in the body use SLAM as a receptor, and only a small minority may also use CD46. We titrated on SLAM-positive and -negative cells the viruses present on throat swabs from several measles patients. The results showed that most samples produced numerous plaques on SLAM-expressing Vero cells, but none (less than the detection limit) on Vero cells (Ono et al., 2001a). Thus, the great majority of MV in the bodies of measles patients use SLAM but not CD46 as a receptor.

The use of CD46 by some MV strains may be considered an \textit{in vitro} adaptation rather than an \textit{in vivo} property of those strains. Such an adaptation can occur easily because a single amino acid substitution at position 481 of the H protein to tyrosine or at position 546 to glycine enables B cell isolated MV strains to interact with CD46 (Shibahara et al., 1994; Bartz et al., 1996; Lecouturier et al., 1996; Rima et al., 1997; Hsu et al., 1998; Xie et al., 1999). Therefore, MV isolation and passage in SLAM-negative cells such as human kidney cells and Vero cells may have selected the viruses capable of using CD46 as a receptor. In fact, glycine at position 546 of the H protein was shown to correlate with the passage history of MV isolates in Vero cells (Woelk et al., 2001). Likewise, the vaccine strains must have adapted to chicken embryo fibroblasts by using a molecule present on them. Figure 1 summarizes our model of the receptor usage of various MV strains.

On the other hand, the use of CD46 as a receptor appears to be more advantageous for MV because distribution of CD46 is ubiquitous, unlike that of SLAM. Thus, some mechanism must operate \textit{in vivo} to suppress the growth of the viruses capable of using CD46 as a receptor. One possible explanation would be that those viruses downregulate CD46 from infected cells, which
are then subject to complement-mediated lysis and are eliminated (Schnorr et al., 1995).

There have been reports that the viruses grow in Vero cells even though their H proteins do not interact with CD46. The recombinant Edmonston MV expressing the H protein of the B cell isolated WTF strain spread in Vero cells, although the parental WTF strain did not (Johnston et al., 1999). A PBMC-isolated strain, which uses SLAM but not CD46 as a receptor, was successfully adapted to Vero cells without the acquisition of the ability to interact with CD46 (Kouomou and Wild, 2002). Another study reported that there was no sequence difference in the H gene between MV strains isolated in B95a and in Vero cells from the same measles patient (Takeuchi et al., 2000). In all these cases, the viruses appear to enter Vero cells independently of SLAM and CD46. By using the recombinant MV expressing the green fluorescent protein, we have demonstrated that a B cell isolated MV strain can infect SLAM-negative cells with 2 to 3 log lower efficiency than it does SLAM-positive cells (Hashimoto et al., 2002). The infection is probably mediated by an as yet unidentified receptor other than SLAM and CD46. Vero cells, which lack the type 1 interferon system, may allow efficient MV replication after some adaptation of the virus, even if entry is inefficient.

**STRUCTURE AND FUNCTION OF SLAM**

Human SLAM was originally identified as a cell-surface glycoprotein of a relative mass of 70 kDa found on activated B cells and T cells (Sidorenko and Clark, 1993; Cocks et al., 1995). It is a member of the immunoglobulin superfamily and has two extracellular domains, V and C2 (Fig. 2). Its cytoplasmic domain contains three tyrosine residues that are surrounded by SH2 domain-binding sequences. In fact, SLAM has been shown to associate intracellularly with SH2 domain-containing molecules such as the SLAM-associated protein (SAP; also known as SH2D1A), protein tyrosine phosphatase SHP-2, and inositol phosphatase SHIP (Sayos et al., 1998; Shlapatska et al., 2001). The SLAM gene is located at the human chromosome 1q22-q23.

SLAM is reported to be a self-ligand (Mavaddat et al., 2000). Engagement of SLAM by a monoclonal antibody A12 leads to IL-2-independent T cell expansion and IFN-γ production by activated T cells, including Th2 cells (Cocks et al., 1995; Aversa et al., 1997). Ligation of SLAM with a monoclonal antibody IPO-3 augments B cell proliferation induced by anti-CD40 and IL-4 (Sidorenko and Clark, 1993). Soluble and membrane-bound forms of SLAM induce proliferation and immunoglobulin synthesis by activated human B cells (Punnonen et al., 1997). SLAM signaling also augments CD95-mediated apoptosis of B cells (Mikalap et al., 1999). However, a recent study showed that SLAM signaling might be involved in inhibiting IFN-γ production by T cells, arguing that the effect of A12 was not agonistic but inhibitory on SLAM signaling (Latour et al., 2001). The study also proposed a model for the signal transduction via SLAM in T cells, which involves the SAP, protein tyrosine kinase Fyn, SHIP, and several other adaptor molecules. Mutations in the human SAP gene cause X-linked lymphoproliferative disease (Sayos et al., 1998), patients with which show

![FIG. 1. Receptor usage of MV strains. Wild-type viruses mainly use SLAM as a cellular receptor. Strains obtained through in vitro selection/adaptation are also able to use other receptors, besides SLAM.](image1)

![FIG. 2. Structure of human SLAM. SLAM contains two highly glycosylated immunoglobulin superfamily domains, V and C2. The cytoplasmic tail of SLAM has tyrosine residues surrounded by SH2-domain-binding sequences, to which SAP binds in phosphorylserine-independent and -dependent fashions.](image2)
progressive lymphocyte expansions after primary EBV infection. SAP knockout mice also exhibit increased IFN-γ production and defect in Th2 development (Wu et al., 2001).

Mouse SLAM has a similar structure (about 60% identity at the amino acid level) and function to the human counterpart (Castro et al., 1999), but cannot act as an MV receptor (Ono et al., 2001b), partly explaining the inability of mice to support MV infection. The V domain of human SLAM fused to various transmembrane proteins was able to act as an MV receptor, whereas a chimeric human/mouse SLAM containing the mouse V domain in place of the human V domain could not function as a receptor. The cytoplasmic domain of SLAM was not necessary for the receptor function. Furthermore, the soluble molecules possessing the V domain of human SLAM bound to cells expressing the MV H protein, but not to cells expressing irrelevant envelope proteins (Ono et al., 2001b). Thus, the V domain of human SLAM is necessary and sufficient to interact with the MV H protein and allow MV entry. Similar to other viral receptors belonging to immunoglobulin superfamily, the N-terminal (membrane-distal) domain of SLAM is responsible for the interaction with the virus.

**DISTRIBUTION OF SLAM**

Human SLAM is constitutively expressed on immature thymocytes, CD45RO− memory T cells, and a proportion of B cells, and rapidly induced on all T and B cells following activation (Sidorenko and Clark, 1993; Cocks et al., 1995; Aversa et al., 1997). SLAM is differentially expressed in CD4 T cells. Whereas high levels of SLAM are found in Th1 cells, only small amounts are detectable in Th2 cells (Hamilainen et al., 2000). Among cultured cells, it is expressed on antigen-specific T cell clones and EBV-transformed B cell lines, but not on most T cell and monocyte/macrophage lines. SLAM is not detected on monocytes, granulocytes, and cells from nonlymphoid organs (Sidorenko and Clark, 1993; Cocks et al., 1995; Aversa et al., 1997).

Although such tissue distribution of SLAM is consistent with lymphotropism of MV (Kobune et al., 1996; McChesney et al., 1997; Griffin, 2001), infection of monocytes, another major target in vivo, cannot be explained. We found that although monocytes freshly isolated from PBMC do not express SLAM, its expression is readily induced after stimulation with mitogens or even with MV particles alone (Minagawa et al., 2001). Furthermore, mature dendritic cells, but not immature dendritic cells, also express SLAM (Polacino et al., 1996; Blehemski et al., 2001; Kruse et al., 2001; Ohgimoto et al., 2001). Anti-SLAM antibody blocks infection of activated monocytes and mature dendritic cells with a B cell isolated MV strain (Minagawa et al., 2001; Y. Yanagi et al., unpublished observation). Thus, MV infection of lymphocytes, monocytes/macrophages, and dendritic cells must be mediated by SLAM.

It is generally thought that MV enters through the respiratory route, initially infecting respiratory epithelial cells, and then spread to lymphoid tissues (Griffin, 2001). We, however, suspect that initial targets of MV in the respiratory tract are SLAM-positive cells of the immune system rather than epithelial cells, since the latter cells are not shown to express SLAM. Furthermore, a recent study showed that the Edmonton strain, which can use CD46 as a receptor, does not efficiently enter respiratory epithelial cells through the apical surface where CD46 is abundantly expressed (Sinn et al., 2002).

There have been reports that tumors regressed in children with Burkitt’s lymphoma and with Hodgkin’s disease after measles (Bluming and Ziegler, 1971; Taqi et al., 1981). These observations may be understood in light of SLAM as an MV receptor. It has been known that EBV-transformed B cells express high levels of SLAM (Aversa et al., 1997), presumably explaining regression of Burkitt’s lymphoma. Probably, the tumor cells of the cases with Hodgkin’s disease would also have expressed SLAM and then been destroyed by MV. These examples suggest that the use of MV for oncolytic therapy (Grote et al., 2001) may be readily applicable to SLAM-expressing tumors.

**SLAM AND MV-INDUCED IMMUNOSUPPRESSION**

Infection and subsequent destruction of SLAM+ cells may explain severe immunosuppression and lymphopenia characteristic of measles. Killing activated lymphocytes and monocytes and mature dendritic cells will lead to impairment of innate immunity as well as that of acquired immunity. Furthermore, the finding that memory T cells and Th1 cells express high levels of SLAM nicely explains why patients with measles show suppressed delayed type hypersensitivity responses such as the tuberculin skin test and exhibit the Th2 polarization in cytokine responses during and after measles (Griffin, 2001).

Mere binding of MV particles or envelope proteins to the V domain of SLAM on the cell surface may affect, by mimicking the natural ligand, the signals induced through SLAM, thereby impairing lymphocyte activation (Yanagi et al., 1992). Although the interaction of the H protein with SLAM results in its downregulation from the cell surface (Erlenhoefer et al., 2001; Tanaka et al., 2002), there is presently no evidence that modulation of SLAM signaling indeed occurs. Schneider-Schaulies and colleagues have shown that the cell surface contact of MV glycoproteins induces inhibition of lymphocyte proliferation in vitro (Schlender et al., 1996), where Akt kinase activation is disrupted (Avota et al., 2001). Their data indicate that both the H and the F proteins are required for the inhibition of lymphocyte proliferation and that the
cleavage of the F protein but not membrane fusion is involved (Weidmann et al., 2000). They have not identified the cell-surface molecule(s) on lymphocytes that interact with MV envelope proteins, but reported that it is not SLAM (Erlenhoefer et al., 2001).

Other mechanisms of MV-induced immunosuppression have also been proposed. CD46 cross-linking by MV inhibits IL-12 production by monocytes (Karp et al., 1996), and suppression of IL-12 production was indeed observed in measles patients, supporting the role of MV-CD46 interaction (Atabani et al., 2001). However, the in vivo significance of the finding remains to be determined because the majority of wild-type MV do not seem to interact with CD46. Recently, the nucleoprotein of MV was shown to have an immunosuppressive activity (Marie et al., 2001). It binds to the Fcy receptor on antigen-presenting cells and impairs their ability to stimulate antigen-specific T cell proliferation. All these mechanisms are not necessarily mutually exclusive and may operate together to cause the severe immunosuppression induced by MV.

SLAM AS MORBILLIVIRUS RECEPTORS

The Morbillivirus genus comprises MV, canine distemper virus (CDV), rinderpest virus (RPV), peste des petits ruminants virus, and emerging morbilliviruses of aquatic mammals (phocine, dolphin, and porpoise distemper viruses). All these viruses are highly contagious pathogens that cause devastating diseases in respective host species accompanied by severe immunosuppression and lymphopenia. It has been reported that the marmoset B cell line B95a is a sensitive host for CDV and RPV as it is for MV (Kobune et al., 1991; Kai et al., 1993).

The common tropism and pathology of these viruses suggested to us that SLAM might also act as receptors for morbilliviruses other than MV. We isolated cDNAs encoding canine and bovine SLAMs from PBMC of respective species and demonstrated that CDV and RPV, respectively, use canine and bovine SLAMs as cellular receptors (Tatsuo et al., 2001). Vaccine strains of CDV and RPV, which had been passaged on SLAM-negative cells, were found to use alternative receptors besides SLAM, probably because of in vitro adaptation. Furthermore, we found that the majority of MV, CDV, and RPV strains examined could use any of human, canine, and bovine SLAMs as receptors, albeit with varying degrees of efficiency, suggesting that the structure required for the interaction with morbillivirus envelope proteins may be well conserved among SLAMs of many different species.

Phylogenetic analysis indicates that CDV is the most distantly related to MV and RPV among morbilliviruses. Thus, the finding that the three morbilliviruses use SLAMs as cellular receptors suggests that this property has been maintained from the ancestral morbillivirus. We suspect that most, if not all, members of morbilliviruses use SLAMs of their respective host species as cellular receptors. Morbilliviruses have been grouped together by their sequence relatedness and lack of neuraminidase activity. Now the use of SLAM as a cellular receptor may be included in their characteristic properties.

CONCLUSIONS

We cannot exclude the possibility that there are still other molecules, including CD46, acting in vivo as a cellular receptor for MV. For example, MV infections of epithelial, endothelial, and neuronal cells reported in the literature (Griffin, 2001) may be explained by an alternative receptor(s). Nevertheless, SLAM appears to be the principal receptor for MV (and morbilliviruses in general), accounting for most of MV pathology and pathogenesis. The Edmonston strain has dominated MV research in the past. Since it may not be representative of MV in the body, we should instead use B cell isolated strains for studying the pathogenesis of MV infection. In this regard, a recently developed reverse genetics system based on a wild-type MV strain will prove most fruitful (Takeda et al., 2000). Furthermore, SLAM transgenic mouse, together with available CD46 transgenic models (Mrkic et al., 1998; Oldstone et al., 1999), will serve as a useful animal model to investigate MV pathogenesis and host immune responses.

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