Chapter 4
Measles Virus Glycoprotein Complex Assembly, Receptor Attachment, and Cell Entry

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Contents

Abstract Measles virus (MV) enters cells by membrane fusion at the cell surface at neutral pH. Two glycoproteins mediate this process: the hemagglutinin (H) and fusion (F) proteins. The H-protein binds to receptors, while the F-protein mediates fusion of the viral and cellular membranes. H naturally interacts with at least three different receptors. The wild-type virus primarily uses the signaling lymphocyte activation molecule (SLAM, CD150) expressed on certain lymphatic cells, while the vaccine strain has gained the ability to also use the ubiquitous membrane cofactor protein (MCP, CD46), a regulator of complement activation. Additionally, MV infects polarized epithelial cells through an unidentified receptor (EpR). The footprints of the three receptors on H have been characterized, and the focus of research is shifting to the characterization of receptor-specific conformational changes that occur in the H-protein dimer and how these are transmitted to the F-protein trimer. It was also shown that MV attachment and cell entry can be readily targeted to designated receptors by adding specificity determinants to the H-protein. These

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studies have contributed to our understanding of membrane fusion by the glycoprotein complex of paramyxoviruses in general.

Introduction

This review focuses on the measles virus (MV) glycoprotein complex: we discuss its assembly and how its interactions with different receptors result in cell entry. The MV glycoprotein complex makes the first contact with the host, targeting the infection to specific cells and thus governing tropism and pathogenesis. MV, one of the most contagious human pathogens, is transmitted by aerosols, infecting a new host via the upper respiratory tract (Panum 1939). It has been assumed that MV infects the respiratory epithelium from the luminal side before spreading in lymphatic cells (Griffin 2007; Cherry 2003). However, the identification of the signaling lymphocytic activation molecule (SLAM, CD150) as the primary MV receptor (Tatsuo et al. 2000) and recent work with selectively receptor-blind MV and animal morbilliviruses (Leonard et al. 2008; von Messling et al. 2006) have brought compelling evidence for a new model of MV dissemination postulating that the systemic spread of wild-type MV depends only on infection of SLAM-expressing lymphatic cells, without initial virus amplification in the respiratory epithelium (Fig. 1) (Leonard et al. 2008; de Swart et al. 2007; von Messling et al. 2006; Yanagi et al. 2006). This model implies that MV does not cross the respiratory epithelium immediately after contagion, but only when it leaves the host (Fig. 1). Moreover, it predicts that a virus that does not recognize the unidentified epithelial receptor (EpR) will not be shed. This prediction was confirmed by generating a recombinant EpR-blind MV and demonstrating that it spread in lymphocytes and remained virulent in rhesus monkeys, but importantly was not shed in the respiratory tract (Leonard et al. 2008). The protein initially identified as the vaccine (Edmonston) strain receptor, the membrane cofactor protein (MCP, CD46), a ubiquitous regulator of complement activation, seems to be of minor relevance for wild type MV infections (see the chapter by Y. Yanagi et al., this volume), and the ability of the vaccine strain to use CD46 partially explains its attenuated phenotype (Condack et al. 2007; Schneider-Schaulies et al. 1995).

Glycoprotein Complex Assembly

Understanding how the glycoprotein complex is assembled is important for the characterization of the subsequent mechanisms of receptor-binding and cell entry. The MV envelope covers particles ranging in diameter from 120–300 nm (Armstrong et al. 1982; Casali et al. 1981). The envelope is traversed by the hemagglutinin (H) and fusion (F) glycoproteins, which form spikes that extend about 10–15 nm from the surface of the membrane (Fig. 2). It surrounds the nucleocapsid core, which

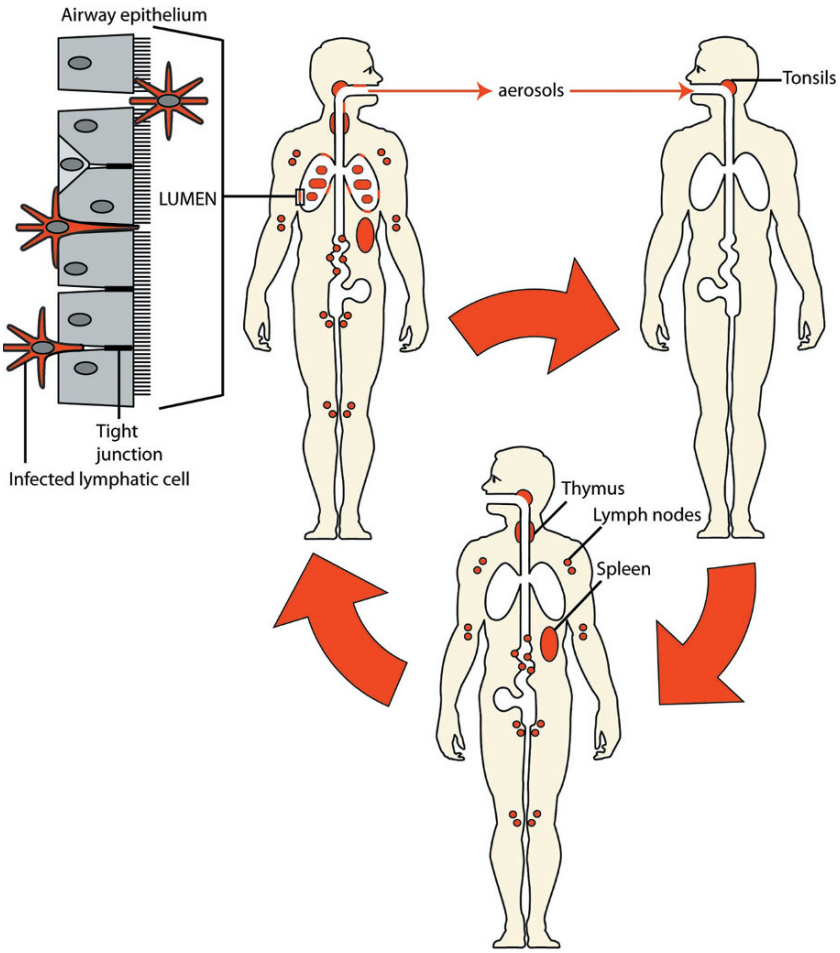

Fig. 1 Disease mechanism of MV. MV is transmitted by aerosol and respiratory secretions. Primary infection may start in SLAM-expressing lymphatic cells in the tonsils (*top right*) and rapidly disseminate to all lymphatic organs (*bottom*). Destabilization of the respiratory epithelium through infected lymphatic cells contacting EpR (*inset, top left*) may result in epithelial crossing of these cells, coughing, and contagion (*top center*)

typically includes several genomes (Rager et al. 2002) tightly encapsidated by a helically arranged nucleocapsid (N) protein. Also associated with the genome are two other proteins, the polymerase (L) and a polymerase cofactor (phosphoprotein, P), all of which together form a replicationally active ribonucleoprotein (RNP) complex (see the chapter by B.K. Rima and W.P. Duprex, this volume). The MV RNP is condensed by the matrix (M) protein (Cathomen et al. 1998), which is hydrophobic and interacts with cell membranes, forming leaflet structures at the inner side of the viral envelope (Fig. 2, bottom). It may mediate the contacts between the cytoplasmic tails of the F and H glycoproteins and the RNP complex.

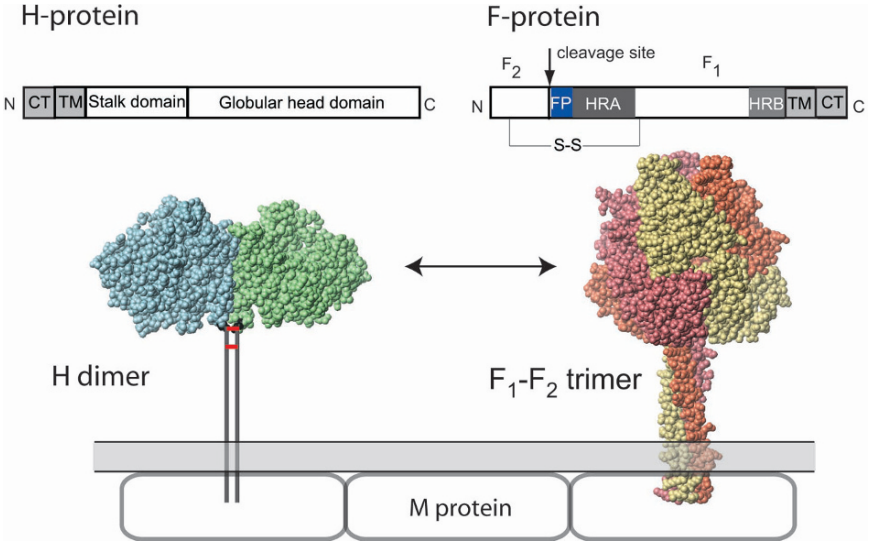

Fig. 2 The MV envelope glycoproteins. *Top left:* Schematic of the MV H-protein. *CT*, cytoplasmic tail; *TM*, transmembrane segment; *N* and *C*, N- and C-terminus, respectively. *Top right:* Schematic of the MV F-protein. The disulfide bond (S-S) holding cleavage fragments F_1 and F_2 is indicated. *FP*, fusion peptide; *HRA*, *HRB*, heptad repeats A and B, respectively. *Center and bottom:* Quaternary structure and interactions of the H- and F-proteins. A space-filling representation of the crystal structure of the MV H dimer (Hashiguchi et al. 2007) and the PIV5 F trimer (Yin et al. 2006) is shown. PIV5 is a paramyxovirus related to MV. The stalk, TM, and CT regions of the H-protein are represented by *two vertical lines* with the two disulfide bonds that hold the H dimer together represented by *horizontal red lines*. The two monomers of the H-protein dimer and the three monomers of the F-protein trimer are shown in different colors for clarity. The interactions of the H- and F-proteins (*double-headed arrow*) are thought to be mediated via the head and stalk domains. The M protein (*clear boxes*) interacts with the membrane (*gray box*) and the CTs of the H- and F-proteins

Here we discuss first the biosynthesis and intercellular transport of the H-protein dimer and F-protein trimer and then the interactions of the H and F envelope oligomers leading to the assembly of fusion-competent glycoprotein complexes.

The Attachment Protein Hemagglutinin Dimer

Receptor attachment of MV and the other morbilliviruses is mediated by the H-protein that has, in addition, a fusion-support function: co-expression of MV H with F is required for fusion (Cattaneo and Rose 1993; Wild et al. 1991). The H-protein binding to SLAM, or other receptors, provides the activation energy to trigger the F-protein to carry out membrane fusion (Navaratnarajah et al. 2008; Yin et al. 2006). Unlike other members of the *Paramyxoviridae*, the morbillivirus H-proteins do not have neuraminidase activity, since H does not bind sialic acid (Hashiguchi et al. 2007).

MV H is a 617 amino acid (78-kDa) type II transmembrane glycoprotein, which is comprised of an N-terminal cytoplasmic tail, a membrane-spanning domain, and an extracellular membrane-proximal stalk region connected to a large C-terminal globular head (Fig. 2, linear map on the top left) (Alkhatib and Briedis 1986). Receptor-binding residues have been mapped to this head domain (Leonard et al. 2008; Navaratnarajah et al. 2008; Tahara et al. 2008; Masse et al. 2004; Vongpunsawad et al. 2004; Hsu et al. 1998). The hemagglutinin-neuraminidase (HN) receptor attachment proteins of other paramyxoviruses exist as tetramers at the viral surface, consisting of a dimer of two disulfide linked homodimers (Lamb 1993). However, the full length MV H only forms dimers (Plemper et al. 2000), and the head domain of H was also crystallized as a dimer (Hashiguchi et al. 2007). We thus refer to the H oligomeric form simply as a dimer. Two disulfide bonds are formed by cysteine residues (C139 and C154) located at the C-terminal end of the stalk domain, just below the globular head (Fig. 1, center, red lines) (Hashiguchi et al. 2007; Plemper et al. 2000).

The crystal structure of the MV H-protein globular head domain was recently reported by two groups (Colf et al. 2007; Hashiguchi et al. 2007). Hashiguchi et al. (2007) reported a dimeric structure (Fig. 2, center left) while Colf et al. (2007) published a monomeric structure. The head domain of MV H (residues 154-607) exhibits a β-propeller structure comprised of six anti-parallel β-sheets (β-sheets 1–6) organized as a superbarrel. This structure most closely resembles the human parainfluenza virus 3 (hPIV3) HN crystal structure (Colf et al. 2007). The overall fold is similar to the sialidase fold previously determined for the HN proteins of hPIV3, Newcastle disease virus (NDV) and parainfluenza virus 5 (PIV5, formerly known as Simian virus 5) (Yuan et al. 2005; Lawrence et al. 2004; Crennell et al. 2000). However, the 3.9-Å root mean square deviation between the Cα atoms of MV H and hPIV3 HN indicates that the H-protein exhibits considerable structural divergence from these proteins. While other paramyxovirus attachment proteins are globular, MV H-protein exhibits a cube-shaped structure. A significant area of the H-protein is covered with N-linked sugars (attached to N200 and N215) and is thus unavailable for receptor interaction (Hashiguchi et al. 2007). Also apparent from the crystal structure is that, in contrast to other paramyxovirus attachment proteins, the two H-protein molecules making up the homodimer are highly tilted with respect to each other. This becomes important when discussing the location of residues implicated in receptor-specific fusion support (see below) (Navaratnarajah et al. 2008).

The Fusion Protein Trimer

The MV F-protein is a 553 amino acid type I transmembrane glycoprotein (Fig. 2, linear map on the top right) (Richardson et al. 1986). A cleavable 28-residue signal sequence at the N-terminus of the nascent polypeptide directs it to the endoplasmic reticulum. A transmembrane (TM) domain near the C-terminal end anchors it in the membrane, leaving a short 33 amino acid cytoplasmic tail. The F-protein is

synthesized as a precursor polypeptide (F_0, 60 kDa), which trimerizes in the endoplasmic reticulum (Plemper et al. 2001). F_0 is then cleaved by the ubiquitous intracellular protease furin in the trans-Golgi (Bolt and Pedersen 1998; Watanabe et al. 1995). Proteolytic cleavage results in a metastable F-protein (Fig. 2, center right) that consists of a membrane-spanning (F_1, 40 kDa), and a membrane-distal subunit (F_2, 20 kDa), that is assembled into virus particles (Lamb 1993). The large F_1 fragment is anchored to the membrane via the TM domain, while the small F_2 fragment is covalently linked to F_1 by a disulfide bond (Fig. 2, top right). The new N-terminus of the F_1 fragment contains a hydrophobic stretch of amino acids, which comprises the fusion peptide (FP) that is inserted in to the target membrane during fusion. The sequences adjacent to the FP and TM exhibit a 4–3 (heptad) pattern of hydrophobic repeats and are named HRA and HRB, respectively (Fig. 2, top right). HRA and HRB sequences are separated by approximately 250 residues and mutagenesis of the heptad repeats adversely affects fusion (Buckland et al. 1992). The MV F-protein has three N-linked carbohydrate chains, all located in the F_2 subunit, that are necessary for proper proteolytic processing and transport to the cell surface (Hu et al. 1995).

The MV F-protein is classified as a class I fusion protein (Kielian and Rey 2006; Yin et al. 2006). The fusion proteins of retroviruses, coronaviruses, Ebola virus, influenza virus, as well as other paramyxoviruses also belong to this class. Class I fusion proteins mediate membrane fusion by coupling irreversible protein refolding to membrane juxtaposition (Lamb and Parks 2007). This is accomplished by discrete conformational changes of a metastable F-protein structure to a lower energy structure. The F-protein found in virions is referred to as the pre-fusion structure and, as it mediates membrane fusion, the F-protein adopts the post-fusion form. The cleavage of F_0 into F_1–F_2 primes the protein for membrane fusion. Activation of the F-protein results in the insertion of the FP, located in the F_1 subunit, into the target membrane. This is followed by dramatic conformational rearrangements of the F trimer, which result in a transient hairpin intermediate and the subsequent formation of a stable six-helix bundle (6HB) structure in which the HRA peptides form a central three-stranded coiled coil, and the HRB peptides pack in an antiparallel manner into hydrophobic grooves on the coiled-coil surface. As a result of these conformational changes, the FP and TM domain, and thus the target and donor membranes, are now in close proximity to each other (Yin et al. 2006). This eventually leads to the formation of a fusion pore through which the RNP complex can enter the cell.

Hetero-oligomerization of F and H Contributes to Particle Assembly

The M-protein is considered the assembly organizer of paramyxovirus particles, and it was formally shown for MV that M regulates the fusion efficiency of the glycoprotein complex (Cathomen et al. 1998). The M-protein assembles in lattice-like

structures at the inner side of the plasma membrane and binds to the cytoplasmic tails of the glycoproteins (Fig. 1, bottom) (Buechi and Bachi 1982), bridging the envelope to the ribonucleocapsid.

Whereas early models of MV assembly assumed that the H- and F-protein ectodomains interact only at the cell surface, it was then shown that the H- and F-proteins form strong lateral interactions already in the endoplasmic reticulum (Plemper et al. 2001). This is in contrast to other paramyxoviruses such as hPIV3 and PIV5, where only minimal amounts of intracellular complexes between HN and F are detected (Corey and Iorio 2007). The H and F glycoprotein complexes are transported through the secretory pathway to the plasma membrane, where they will sustain cell-to-cell fusion or virus particle release. While the exact interaction sites and stoichiometry of H and F in these glycoprotein complexes is yet to be determined, based on the fusion-support capacity of chimeric attachment proteins from different paramyxoviruses and antibody mapping studies, it appears that the stalk and head domains of H are responsible for conferring F specificity (Fournier et al. 1997).

A key question that remains to be addressed in the MV entry process, and indeed for all paramyxoviruses, is how the receptor attachment protein triggers the F conformational changes upon receptor binding. Plemper et al. (2002) observed that the stability of the H-F complex was a modulator of virus-induced cell-to-cell fusion and that destabilizing the H–F interaction results in a significant increase in lateral cell-to-cell fusion. This observation is in agreement with a model proposed for NDV fusion activation, where the pre-fusion state of the F-protein is stabilized by association with the HN-protein and upon receptor attachment the HN–F complex dissociates, thus leading to F activation and fusion (McGinnes and Morrison 2006). Recent results of Mühlebach et al. (2008) highlight the importance of the H–(F_1+F_2) glycoprotein complex for fusion. Mutations that reduced the amount of this complex were less fusogenic, while mutations that increased its availability were more fusogenic (Mühlebach et al. 2008). All these observations are consistent with a role for the H-protein in stabilizing the F-protein prior to fusion. The strong H–F interaction observed in infected cells may be responsible for preventing premature fusion after furin cleavage of the F-protein has converted it to a metastable pre-fusion structure.

Receptor Attachment, Membrane Fusion, and Cell Entry

This section presents a discussion of the H-protein interactions with the two known receptors (CD46 and SLAM) and the putative epithelial cell receptor (EpR). Experiments with CD46 have revealed the importance of a receptor-H protein scaffold as a prerequisite for fusion. Subsequent experiments with SLAM have demonstrated that there are receptor-specific H-protein conformational changes that lead to fusion activation. The footprint of EpR has already been defined on H, even if the identity of this receptor remains unknown. Finally, the ability to retarget

MV to specific receptors via specificity determinants fused to the C-terminus of the H-protein is discussed.

Cell Entry Through CD46: Scaffolding Fusion Through Receptor-H Protein Interactions

The first MV receptor to be identified was the ubiquitous regulator of complement activation, CD46 (see the chapter by C. Kemper and J.P. Atkinson et al., this volume) (Dorig et al. 1993; Naniche et al. 1993). MV adapts to use CD46 by accumulated changes in the H-protein, the most significant of which is an N481Y substitution (Bartz et al. 1996; Lecouturier et al. 1996). The attenuated phenotype and altered tropism of the MV vaccine strain compared to the wild-type strains are partly explained by the use of CD46. Although CD46 is not the principal cellular receptor for the wild-type strain of MV, experiments with this receptor have yielded important insights into the mechanism of fusion activation in MV and other morbilliviruses (Buchholz et al. 1996, 1997).

These experiments provided insights into how the H-protein and a receptor may hold the virus in place before the F-protein trimer unfolds and mediates membrane fusion. Buchholz et al. (1996) demonstrated that increasing the length of the CD46 protein, thereby effectively increasing the distance between the viral and cell membranes, enhances binding but reduces fusion. The increase in virus binding can be explained by the alleviation of steric hindrance as the attachment site on the receptor is moved further away from the cell surface. The fact that the fusion efficiency decreased as a result of this indicates that the viral and cellular membranes need to be at a certain distance from each other for fusion to occur. This distance is probably defined by the need for the fusion peptide to efficiently insert into the target cell membrane. Another key finding was that the CD46 receptors with increased length had a dominant-negative effect on fusion when co-expressed with functional CD46 receptors, even at an unfavorable molar ratio. This is indirect evidence for the existence of a MV fusion complex in which multiple H-protein dimers bind CD46 and form a scaffold surrounding multiple F-protein trimers. Co-expressing CD46 molecules of different length would result in an irregular scaffold that would be unable to support fusion, thus explaining the dominant-negative effect.

The extracellular domain of CD46 consists of four complement control protein domains (CCP1–4) (Fig. 3D, top left). The MV binding site has been localized to the membrane distal CCP1 and CCP2 domains (Casasnovas et al. 1999; Devaux et al. 1997; Manchester et al. 1997). Specifically, the region comprising CD46 residues 37–59 is involved in H-protein interactions (Buchholz et al. 1997) and mutation of R59 in the CCP1 domain interferes with viral fusion and CD46 downregulation (Hsu et al. 1999). On the H-protein, V451 and Y481 have been identified as CD46-dependent fusion-support residues (Lecouturier et al. 1996). Binding studies with the soluble extracellular domain of the H-protein and soluble receptors demonstrated the importance of the tyrosine at position 481 (Navaratnarajah

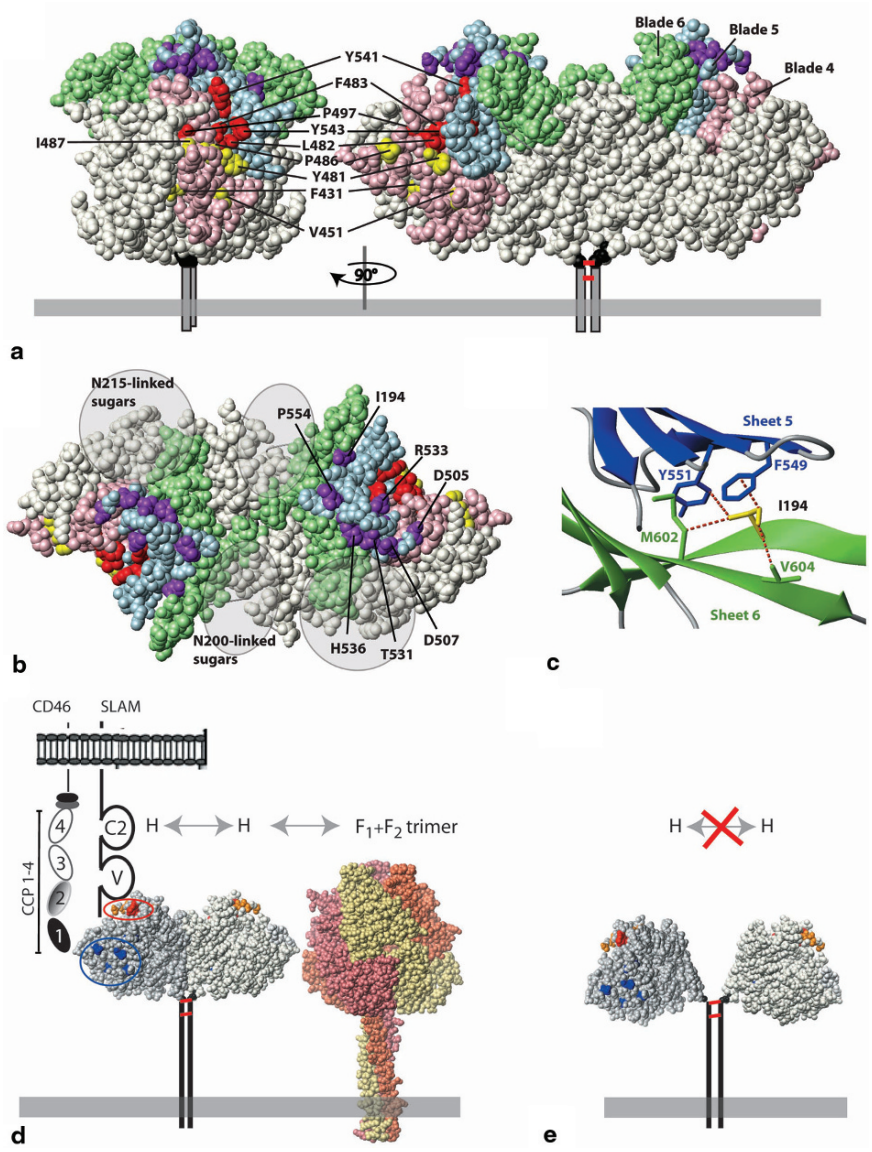

Fig. 3 a–e Receptor footprints on the H-protein dimer and a model for fusion activation. **A** Side view of the MV H-protein homodimer crystal structure depicted as a space-filling model. Residues 1-153, not present in the crystal structure and comprising the cytoplasmic tail, transmembrane, and stalk regions are represented as *vertical gray boxes*. The *two red lines* represent the C154-C154 (residues colored *black*) and C139-C139 disulfide bonds that link the two monomers in the homodimer. The membrane is illustrated as a *horizontal gray box*. β-Propeller blades 4, 5, and 6 are surface shaded *pink*, *blue*, and *green*, respectively. SLAM-, CD46-, and EpR-specific residues are shaded *purple*, *yellow*, and *red*, respectively. **B** Top view of the H homodimer generated by rotating the view depicted in **A** (*right panel*) 90° around the x-axis. The regions occluded by the N-linked oligosaccharide chains are indicated by *gray ovals*. The color coding is identical to **A**.

et al. 2008; Hashiguchi et al. 2007). The wild-type strain of MV carries an asparagine at this position and does not interact with CD46 in surface plasmon resonance-based binding assays. However, when the asparagine was substituted by tyrosine, the soluble H-protein showed an appreciable interaction with CD46.

Figure 3 presents the recently published crystal structure of the H dimer with receptor-specific residues indicated (Navaratnarajah et al. 2008; Hashiguchi et al. 2007). In this structure, the two H monomers tilt oppositely toward the horizontal plane (Fig. 3A, right panel). This orients propeller blades 5 (blue surface) and 6 (green surface) with the SLAM-relevant residues upward toward the target cell (Fig. 3A and 3B). Mapping of the CD46-specific fusion-support residues (F431, V451, Y481, P486, and I487) on to the MV H-protein structure defines a region that involves propeller blade 4 (pink surface) located on the side of the H-dimer (Fig. 3A and 3B, residues shaded yellow). Most CD46-relevant residues are located in the bottom half of the dimer. This site is adjacent to but distinct from the SLAM- and EpR-specific sites (see below).

Cell Entry Through SLAM: Dynamic Interactions and Conformational Changes

SLAM is the primary cellular receptor for the wild-type strains (Erlenhoefer et al. 2001; Hsu et al. 2001; Ono et al. 2001a; Tatsuo et al. 2000). Its tissue-specific expression is consistent with MV lymphotropism and may be the fundament for pathogenesis and immunosuppression (see the chapter by Y. Yanagi et al. this volume, and chapter by S. Schneider-Schaulies and J. Schneider-Schaulies, accompanying volume). SLAM is composed of two immunoglobulin superfamily domains, V and C2, followed by a TM and a cytoplasmic domain (Fig. 3D, top left) (Cocks et al. 1995). The V domain interacts with H (Ono et al. 2001b). Specifically, I60, H61, and V63 are key residues supporting this interaction, forming a putative MV H binding site (Ohno et al. 2003).

Mutagenesis of the H-protein based on structural models has characterized residues I194, D505, D507, Y529, D530, T531, R533, H536, Y553, and P554 as important for SLAM-dependent fusion (Navaratnarajah et al. 2008; Masse et al. 2004; Vongpunsawad et al. 2004). Furthermore, it was shown that mutating

Fig. 3 (Continued) **C** Interactions of I194 with neighboring MV H residues. Closest distances between heavy atoms of I194 and interaction partners are depicted as *dotted red lines*. I194 has van der Waals contacts with Y551 and F549 on sheet 5 (*blue*) and with M602 and V604 on sheet 6 (*green*). **D** Close apposition of H dimers and F trimers before fusion activation. Schematics of the SLAM and CD46 receptors are illustrated next to their respective binding sites on the H-protein (*red circle*, SLAM; *blue circle*, CD46). Complement control protein domains 1–4 (CCP 1–4) of CD46 and immunoglobulin domains V and C2 of SLAM are indicated. Prior to receptor binding, the H dimer is closely associated with the F trimer. **E** H-protein dimer destabilization after receptor-binding. Conformational changes of the H dimer may disrupt the H-(F_1+F_2) interaction, resulting in fusion activation

I194 ablates SLAM-binding (Navaratnarajah et al. 2008). In contrast, mutating Y529, D530, R533, and P553 (termed the β-sheet 5 quartet), while interfering with SLAM-dependent fusion, does not affect SLAM binding. The location of these residues is visualized in Fig. 3 on the crystal structure of the H-protein ectodomain dimer. The SLAM relevant residues (purple) are centrally located on the top of the H-protein homodimer.

The results of assays measuring SLAM-dependent fusion-support and SLAM-binding gave insights in to H-SLAM binding dynamics, which may be based on sequential H-protein conformational changes. We know that the initial SLAM interaction is influenced by I194, and Fig. 3C illustrates how this residue, located on one β-strand of propeller blade 6 (green), engages in van der Waals interactions (red dashed lines) with M602 and V604 on another β-strand of propeller blade 6, as well as with F549 and Y551 on a β-strand of propeller blade 5 (blue), stabilizing this interface. Thus, I194 may maintain a conformation of the H-protein conducive to SLAM-binding, rather than directly interacting with the receptor. The fact that four of the residues (β-sheet 5 quartet) are essential for SLAM-dependent fusion but not for SLAM binding proves that H-protein conformational changes can be receptor-specific (Navaratnarajah et al. 2008). It also implies that alternative pathways of H-protein conformational changes can converge to trigger F-protein unfolding and membrane fusion.

Cell Entry Through EpR: The Footprint

Lack of SLAM expression in epithelial cells suggests that another receptor exists (de Swart et al. 2007; von Messling et al. 2006; Yanagi et al. 2006). While the identity of the putative epithelial cell receptor (EpR) has remained elusive, two groups have independently mapped H-protein residues specifically sustaining EpR-dependent fusion (Leonard et al. 2008; Tahara et al. 2008). These studies identified H-protein residues L482, F483, P497, Y541, and Y543 (Fig. 3, red residues) located in an area distinct from that defined by the SLAM-interacting residues. They define a nonpolar valley running between propeller blades 4 and 5 (Fig. 3A, left panel). The nonpolar side chains of L482, F483, and P497, together with the uncharged polar side chains of Y541 and Y543, flank this valley situated between the SLAM- and the CD46-binding sites. The hydroxyl group of tyrosine 543 tops one of the ridges. Thus, uncharged polar and nonpolar residues govern the H–EpR interaction. It is not known which EpR-relevant residues are directly involved in receptor binding. Residues L482, F483, P497, Y541, and Y543 are 13%, 14%, 6%, 28%, and 17% solvent-exposed, respectively. Thus, the minimally exposed proline 497 may conduct a receptor-dependent conformational change. As for tyrosine 543, the hydroxyl group on the aromatic ring may play a central role in EpR binding: replacing it with phenylalanine, which differs from tyrosine by carrying a hydrogen atom in place of the hydroxyl group at this position, resulted in loss of EpR-specific fusion-support while maintaining SLAM-dependent function (Leonard et al. 2008).

Cell Entry Through Designated Receptors: A Compliant Membrane Fusion System

MV can be readily targeted to enter cells through a designated receptor by adding a specificity determinant to the C-terminus of the H-protein. First demonstrated for the epidermal growth factor receptor (Schneider et al. 2000), MV has since been targeted to a variety of different cell surface molecules (see the chapter by S. Russell and K.W. Peng, accompanying volume). Table 1 shows that receptor choice is not a limitation for membrane fusion. MV has been modified to utilize a broad range of cell surface antigens for cell entry, irrespective of their structure or function. These proteins can span the membrane only once, in type I or type II orientations, or several times, or even be anchored to the membrane by a glycosphingolipid. They can be monomeric, homo-oligomeric, or hetero-oligomeric, and have a wide range of functions, as well as cell-type specificity of expression. Equally important is the ability of the virus to accommodate the corresponding ligands, which range from the relatively small epidermal growth factor ligand to the significantly larger single-chain antibodies.

The ability to retarget MV entry, including detargeting of the natural receptors, coupled with its inherent oncolytic nature, has provided new perspectives for the development of MV-based phase I or phase I/II clinical trials of ovarian cancer, glioma, and myeloma (Liu et al. 2007) (see the chapter by S. Russell and K.W. Peng, accompanying volume, for an in-depth analysis). Two other developments enhancing specificity and efficacy of oncolytic MV are the use of cancer-specific proteases to activate the F-protein (Springfeld et al. 2006) and arming of the virus by prodrug convertases (Ungerechts et al. 2007a, 2007b). By altering the location of the furin cleavage site of F through addition of hexameric sequences recognized by matrix metalloproteinases, it was possible to generate a recombinant virus that only spreads in cancer cells expressing these proteases. Moreover, arming of MV by prodrug convertases sustains oncolytic efficacy even in mouse tumors set in fully immunocompetent mice (Ungerechts et al. 2007b). In particular, arming MV with the prodrug convertase purine nucleoside phosphorylase (PNP) is being sought in the context of non-Hodgkin lymphoma treatment, because PNP converts fludarabine phosphate, a drug used in combination with other chemotherapeutics to treat lymphoma (Ungerechts et al. 2007a). Finally, MV envelope proteins have been used to target retroviruses and lentiviruses, further extending the therapeutic potential of these vectors (Funke et al. 2008).

Model for MV H-Mediated Fusion Activation

Based on the data discussed above, the following model of MV-induced membrane fusion can be postulated. As shown in Fig. 3D, H dimers are associated with F_1–F_2 trimers prior to receptor attachment (Plemper et al. 2001). First, upon receptor binding the H-protein dimer creates a scaffold with the receptor (Fig. 3D, left), locating

Table 1 Measles virus natural and designated receptors

Synonyms	Predominant expression site	Size (kDa)	Transmembrane organization	Quaternary structure	Function	Reference
Natural receptors						
CD150 (SLAM)	Lymphatic cells	70–95	Type I	Monomer	Adhesion/signaling	
CD46 (MCP)	Ubiquitous	55–65	Type I	Monomer	Regulator of complement activation	
Designated receptors						
CD20	B lymphocytes	34	Tetraspanin	Homo-oligomers	Signaling/cell activation	Bucheit et al. 2003
CD38	Leukocytes	45	Type II	Homodimer	ADP-ribosyl cyclase/ signaling	Peng et al. 2003
CEA	Early development transformed cells	180	GPI-anchor	–	Adhesion	Hammond et al. 2001
EGFR (ErbB–1, HER1)	Lung, GI tract, breast epithelium	170	Type I	Monomer	Signaling	Nakamura et al. 2004
EGFRvIII	Tumor cells	140	Type I	Monomer	Signaling	Nakamura et al. 2005
IGFR	Ubiquitous	130/95	Type I (β chain)	Heterotetramer	Signaling	Schneider et al. 2000
Intergrin avβ3	OEFMP[a]	160/85	Type I/type I	Heterodimer	Adhesion/signaling/ internalization	Hallak et al. 2005

[a]Osteoclasts, endothelial cells, fibroblasts, macrophages, platelets

the F trimer at an appropriate distance for the fusion peptide to reach the target membrane (Buchholz et al. 1996). Second, the H-receptor interaction triggers F to initiate the fusion process. As shown in Fig. 3E, the trigger may be simply the destabilization of the H-(F_1+F_2) interaction or it may entail a more complex set of H-protein conformational changes. As has been suggested for NDV (Ludwig et al. 2008; McGinnes and Morrison 2006), the interaction of the H-protein with F_1+F_2 may be essential for stabilizing the metastable pre-fusion form of the F-protein, thus preventing premature fusion (Plemper et al. 2002). For PIV5, it has been suggested that the interaction of the tetrameric attachment protein with sialic acid receptors destabilizes the tetramer, which in turn leads to changes in the interaction with F, which triggers F to mediate membrane fusion (Yuan et al. 2005). An analogous model based on dimer destabilization can be adopted for MV fusion: the interaction of MV H with its receptor may destabilize the H dimer (Fig. 3E), which in turn will destabilize the H–(F_1+F_2) interaction, resulting in F activation. In retrospect, the fact that many different specificity domains displayed on the H-protein can elicit fusion through targeted receptors can be rationalized by the availability of alternative pathways of H-protein conformational changes eliciting membrane fusion (Navaratnarajah et al. 2008).

Perspectives

In the last 15 years, the identification of two MV receptors, together with mutagenesis studies based on structural models of the MV H-protein, have supported the characterization of the H–receptor interactions that govern tropism and pathogenesis. The recently determined crystal structure of MV H allows accurate planning of the next phase of experimentation, which aims at understanding the molecular dynamics of receptor binding and fusion activation. More crystal structures of individual molecules or quaternary complexes are needed. However, these structures will represent only one possible conformation, while proteins and protein complexes are constantly moving in solution as a result of thermal energy. Further elucidation of the mechanism of fusion activation and cell entry will thus depend on our ability to study conformational changes through conformation-specific antibodies, sophisticated fusion assays, and computational modeling techniques based on molecular dynamic simulations.

In another new avenue of research, the insights gained by the mutagenesis studies on the determinants of MV tropism in the H-protein have yielded selectively receptor-blind viruses. These new tools have been used for basic research studies on the mechanisms of virulence and pathogenesis, and will be integrated in the next generation of oncolytic virotherapy clinical protocols. While measles is under control and may soon be eradicated, the study of MV biology remains of central importance for the development of MV-based replicating therapeutics to treat cancer, and for the generation of multivalent vaccines (see the chapter by M. Billeter and S. Udem, this volume).

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