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Differential potential for envelope glycoprotein-mediated steric shielding of host cell surface proteins among filoviruses

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ABSTRACT

The viral envelope glycoprotein (GP) is thought to play important roles in the pathogenesis of filovirus infection. It is known that GP expressed on the cell surface forms a steric shield over host proteins such as major histocompatibility complex class I and integrin β1, which may result in the disorder of cell-to-cell contacts and/or inhibition of the immune response. However, it is not clarified whether this phenomenon contributes to the pathogenicity of filoviruses. In this study, we found that the steric shielding efficiency differed among filovirus strains and was correlated with the difference in their relative pathogenicities. While the highly glycosylated mucin-like region of GP was indispensable, the differential shielding efficiency did not necessarily depend on the primary structure of the mucin-like region, suggesting the importance of the overall properties (e.g., flexibility and stability) of the GP molecule for efficient shielding of host proteins.

Introduction

Filoviruses (viruses of the genera *Marburgvirus* and *Ebolavirus* in the Family *Filoviridae*) are enveloped, negative-stranded RNA viruses. Filovirus infection causes severe hemorrhagic fever in human and non-human primates (Feldmann and Geisbert, 2011). To date, there is one known species in the genus *Marburgvirus* consisting of two distinct viruses, Marburg virus (MARV) and Ravn virus. In contrast, five viruses (Ebola virus, Sudan virus, Taï Forest virus, Bundibugyo virus, and Reston virus) are recognized within the genus *Ebolavirus*, representing the distinct virus species, *Zaire ebolavirus*, *Sudan ebolavirus*, *Taï Forest ebolavirus*, *Bundibugyo ebolavirus*, and *Reston ebolavirus*, respectively. Differential pathogenicity has been suggested among filoviruses (Geisbert and Hensley, 2004; Mahanty and Bray, 2004). Ebola virus (EBOV) within the species *Zaire ebolavirus* is thought to be the most pathogenic among the viruses in the genus *Ebolavirus* with case-fatality rates up to 90%, whereas Reston virus has never caused lethal infection in humans. Among Marburg viruses, the Angola strain caused the largest outbreak with the highest case fatality rate (90%) among Marburg viruses. While there was another outbreak in Durba in 1998–2000 in which the case fatality rate was 83% (Bausch et al., 2006), it is noted that in most of earlier outbreaks of Marburg hemorrhagic fever case fatality rates did not exceed 50% (Smith et al., 1982; Bausch et al., 2008), and that macaques experimentally infected with the Angola strain died after a rapidly progressive illness if compared with other viruses such as the strain Musoke (Musoke) tested in the previous experiments (Daddario-DiCaprio et al., 2006; Geisbert et al., 2007). Thus, it could be suggested that the Angola strain is more pathogenic than the Musoke strain, although statistically significant data are not available for the Musoke strain, due to low case numbers. However, the molecular basis explaining the differential pathogenicity of filoviruses remains elusive, although previous studies suggested that the viral envelope glycoprotein (GP) may play important roles (Geisbert and Hensley, 2004; Matsuno et al., 2010; Simmons et al., 2002; Takada et al., 2004; Yang et al., 2000).

Filovirus GP is the only spike protein and is responsible for virus entry into host cells. EBOV and MARV GP undergoes proteolytic cleavage by host proteases such as furin, resulting in

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production of two subunits, GP1 and GP2, which are linked by a single disulfide bond (Jeffers et al., 2002; Volchkov et al., 1998; Volchkov et al., 2000). GP1 contains a putative receptor-binding region and mucin-like region (MLR) that has a number of potential N- and O-linked glycosylation sites (Dube et al., 2009; Kuhn et al., 2006). GP2 has a transmembrane domain, cytoplasmic tail, and internal fusion loop (Sanchez et al., 2007; Weissenhorn et al., 1998).

Expression of EBOV GP in cultured cells results in loss of cell–cell interaction as well as cell rounding and detachment of cells from the substrate (Chan et al., 2000; Takada et al., 2000; Yang et al., 2000). Though this can be observed in various types of cells, the subsequent results of GP expression are different among cell types (Simmons et al., 2002). While human cardiac microvascular endothelial cells were reported to undergo anoikis and detachment-mediated apoptosis upon GP expression (Ray et al., 2004), transient expression of GP did not cause death in human embryonic kidney 293T cells (Simmons et al., 2002).

GP-mediated downregulation of the host cell surface proteins such as integrin β1 was proposed to be the molecular mechanism for morphological changes of host cells (Simmons et al., 2002; Takada et al., 2000). However, a recent study demonstrated that these host proteins were indeed expressed but sterically masked by GP on the cell surface (Francica et al., 2010). It was proposed that the MLR of GP, which is highly glycosylated and spatially occupies a very large region, formed a steric shield over host proteins including integrin β1, major histocompatibility complex class I (MHC I), and other immune molecules on the surface of GP-expressing cells, which might result in abrogation of cell adhesion and prevention of interaction between lymphocytes and infected cells.

In this study, to investigate the possible contribution of GP-mediated steric shielding to the pathogenicity of filoviruses, we compared the shielding effects among filovirus strains having different pathogenicities and found a correlation between the shielding effects and their pathogenic potential. In addition, we mapped the GP regions responsible for the different shielding effects observed among the viruses tested.

Results

Comparison of the shielding effects between viruses of the genus Ebolavirus

It has been demonstrated that the MLR forms a steric shield over integrin β1 and MHC I on the surface of GP-expressing cells (Francica et al., 2010). To compare this effect between viruses with differential pathogenicity, HEK293T cells were transiently transfected with plasmids expressing GP of a strain Mayinga-76 (Zaire) or a strain Reston-89 (Reston) and analyzed by flow cytometry with probing antibodies to integrin β1 (MAB17-029) and MHC I (Fig. 1). The steric shielding was expected to be observed as decreased cell surface expression levels of these host proteins due to the sterically hindered antibody access to the proteins. A prominent shielding effect for integrin β1 was observed on Zaire GP-expressing cells compared with that of Reston GP (Fig. 1A, left panel). On the other hand, MHC I was comparably shielded by Zaire and Reston GPs and this molecule was almost undetectable on the cells expressing these GPs (Fig. 1B). We quantified the expression levels of both host proteins by calculating the relative means of fluorescence intensity (MFI) and confirmed the significantly different shielding effects between Zaire and Reston GPs (Fig. 1C). It should be noted that integrin β1 was detected normally on the surface of GP-expressing cells when cells were stained with another antibody (MAB1965) whose epitope is likely different from that of MAB17-029 (Fig. 1A, right panel), confirming that the reduced detection level of cell surface integrin β1 by MAB17-029 was not due to reduced expression (i.e., down-regulation) of this molecule. Furthermore, we confirmed by Western blotting that overall intracellular expression levels of these host proteins were not affected by the expression of GPs (Fig. 1D).

Comparison of the shielding effects between MARV strains

To examine whether the shielding effect was similarly observed upon MARV GP expression, we selected two strains, Angola and Musoke, which likely have differential pathogenicity for humans and nonhuman primates (Daddario-DiCaprio et al., 2006; Geisbert et al., 2007). These MARV GPs also shielded integrin β1 and MHC I molecules on cell surfaces, suggesting that the steric shielding effect is a common phenomenon in filovirus GP-expressing cells (Fig. 2A, B, and C). Consistent with the expression of Zaire and Reston GPs, the MHC I molecule was more markedly shielded than integrin β1. Interestingly, Angola GP showed more prominent shielding effects for both proteins than Musoke GP. Similarly to Zaire and Reston GP-expressing cells, intracellular expression levels of these host proteins were not affected by the expression of MARV GPs (Fig. 2D).

Role of the MLR structure in the steric shielding

To ascertain whether the highly glycosylated MLR played an essential role for GP-mediated shielding effects, we constructed MLR-deletion mutant GPs (ZΔmuc and AΔmuc) and analyzed their shielding effects by comparing them with the respective wild-type GPs. As expected, reduced shielding effects were observed on the cells expressing MLR-deletion GPs (Fig. 3). We then focused on the role of the MLR in different shielding effects between filovirus strains. Amino acid sequence comparison between Zaire and Reston GPs indicates that the similarity of their MLRs is approximately 16%. Although there is a relatively high amino acid similarity between Angola and Musoke GPs (86%), the numbers of potential O-glycosylation sites vary between these GPs (i.e., Angola GP has more potential O-glycosylation sites than Musoke GP), suggesting that the steric shielding effect is potentially dependent on the primary structure of the MLR. To address this hypothesis, we constructed chimeric mutant GPs whose MLRs were swapped between viruses (ZRZ, RZR, AMA, and MAM) (Fig. 4A and B) and analyzed their shielding effects together with wild-type GPs (Fig. 4C and D). As compared with wild-type Zaire GP, slightly decreased and comparable effects for integrin β1 and MHC I, respectively, were observed on cells expressing ZRZ. Unexpectedly, only small shielding effects on integrin β1 and MHC I were observed on cells expressing RZR. Similarly, swapping of the MLR of MARV GPs (AMA and MAM) did not reverse the phenotype. Taken together, these results indicated that the MLR was required for efficient steric shielding, but its primary structure was not essential for the differential effects between Zaire and Reston or Angola and Musoke GPs.

Identification of the GP region required for efficient steric shielding

To further investigate which region of GP was involved in the efficiency of steric shielding, chimeric mutants between Zaire and Reston GPs (ZZR, ZRR, RRZ, and RZZ) or Angola and Musoke GPs (AAM, AMM, MMA, and MAA) were constructed (Fig. 5A and B). Then the efficiency of steric shielding caused by these mutant GPs was compared with the condition in wild-type GPs (Fig. 5C and D). While ZZR showed a shielding effect on integrin β1 as strong as that of wild-type Zaire GP, the other chimeric mutants between Zaire and Reston GPs showed comparable or lesser shielding effects compared to wild-type Reston GP. MHC I was almost undetectable on cells expressing