Viral Apoptotic Mimicry Party: P.S. Bring Your Own Gas6

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In the evolutionary battle between virus and host, viruses have developed numerous strategies to subjugate indispensable cellular functions. In this issue of *Cell Host & Microbe*, Morizono and colleagues (Morizono et al., 2011) describe how viruses hijack host apoptotic clearance machinery for entry. A host factor called Gas6 enhances infection by bridging virus phosphatidylserine to the clearance receptor Axl.

Endocytosis is a highly regulated process by which cells interact with and sample their environment. It is essential for proper cell function, tissue development and homeostasis, and pathogen clearance. For instance, under normal cellular conditions the lipid phosphatidylserine (PS) is rigorously maintained on the inner leaflet of the plasma membrane. However, when cells undergo apoptosis (programmed cell death), they expose PS on their surface. This exposed PS serves as the major "eat me" signal for the endocytic internalization and elimination of apoptotic cells. Like all forms of endocytosis, the removal of apoptotic debris requires the engagement of specific receptors. Several PS receptors required for apoptotic clearance have been identified (Zhou and Yu, 2008). These receptors come in two flavors: those that directly recognize PS on the target membrane, and those that require an "opsonin" or bridging molecule. Opsonins are secreted proteins that literally link the PS in the target membrane with its cognate receptor. One family of PS receptors that require opsonins is the TAM (Tyro3, Axl, Mer) receptor tyrosine kinases (Lemke and Burstyn-Cohen, 2010). The TAM receptors rely on the bridging molecules serum protein S or Gas6 (Ishimoto et al., 2000). Protein S and Gas6 are proteins composed of two domains: an amino-terminal glutamic acid rich domain that recognizes PS, and at the carboxyl terminus two laminin G domains that bind and activate the TAM receptors to promote endocytosis of PS containing particles.

To infect successfully, the first barrier a virus has to overcome is entry into host cells. As opportunist pathogens, viruses have devised many strategies to take advantage of existing endocytic mechanisms, so it is perhaps no surprise that they have subjugated the apoptotic waste removal system as a way of entering host cells. Virus "apoptotic mimicry," the exposure of PS on a pathogen surface to induce virus uptake or evade the host immune system, was first proposed for hepatitis B virus (Vanlandschoot and Leroux-Roels, 2003). Apoptotic mimicry was subsequently shown for vaccinia, pichinde, cytomegalo, and lassa fever viruses, and possibly HIV, ebola, and marburg (Callahan et al., 2003; Mercer and Helenius, 2008; Shimojima et al., 2006; Soares et al., 2008). Vaccinia, HIV, and pichinde viruses have been shown to concentrate PS in their envelope membrane. That viral PS is required for efficient entry and infection suggests that PS receptors are used by these viruses. However, no direct link between virus PS and cellular PS receptors has been identified. In this issue of *Cell Host & Microbe*, Morizono and colleagues provide a link between viral apoptotic mimicry and cellular PS receptors. They demonstrate that Gas6 enhances infection by lentivirus vectors and vaccinia virus (VACV) by bridging PS in the viral envelope with the apoptotic clearance receptor Axl.

Residual transduction of pseudotyped adeno- and lentivirus vectors that is observed even when the receptor-binding activity is ablated can hinder the use of these vectors for gene therapy. While investigating the cause of this residual transduction, Morizono and colleagues found that a factor present in fetal calf serum enhanced transduction. Purification of this "enhancement factor" led to its identification as bovine protein S. Additional studies demonstrated that its human homologs, the PS bridging molecules human protein S and Gas6, also enhanced virus binding and transduction. Using annexin V (ANX5), another PS-binding protein, they could show that these lentivirus vectors have PS in their envelope. Masking of the lentivirus vector PS with ANX5 blocked Gas6-mediated enhancement of vector binding and transduction. Intriguingly, while Gas6 could enhance vector transduction regardless of the viral envelope protein used for pseudotyping, the level of Gas6 enhancement showed an inverse relationship to the binding efficiency of the different pseudotyped particles. The authors hypothesize that when receptor affinity or avidity is low, apoptotic mimicry might enhance transduction. Thus, apoptotic mimicry may serve as a mechanism for these viruses to broaden their host range or tissue tropism when their natural receptors are low in abundance or not available.

To complete this picture, the authors sought to identify the cognate TAM receptor used for Gas6-mediated enhancement. The TAM receptors (Tyro3, Axl, or Mer) were ectopically expressed in a cell line nonpermissive for lentivirus transduction. Overexpression of Axl and Tyro3, but not Mer, rendered these cells susceptible. Axl was found to be most efficient, and its role as the primary receptor for Gas6-mediated lentivirus transduction was confirmed using anti-Axl blocking antibodies. Gas6-mediated clearance of apoptotic cells relies predominantly on signaling through Mer. The preferential use of Axl for apoptotic mimicry by lentiviral vectors suggests

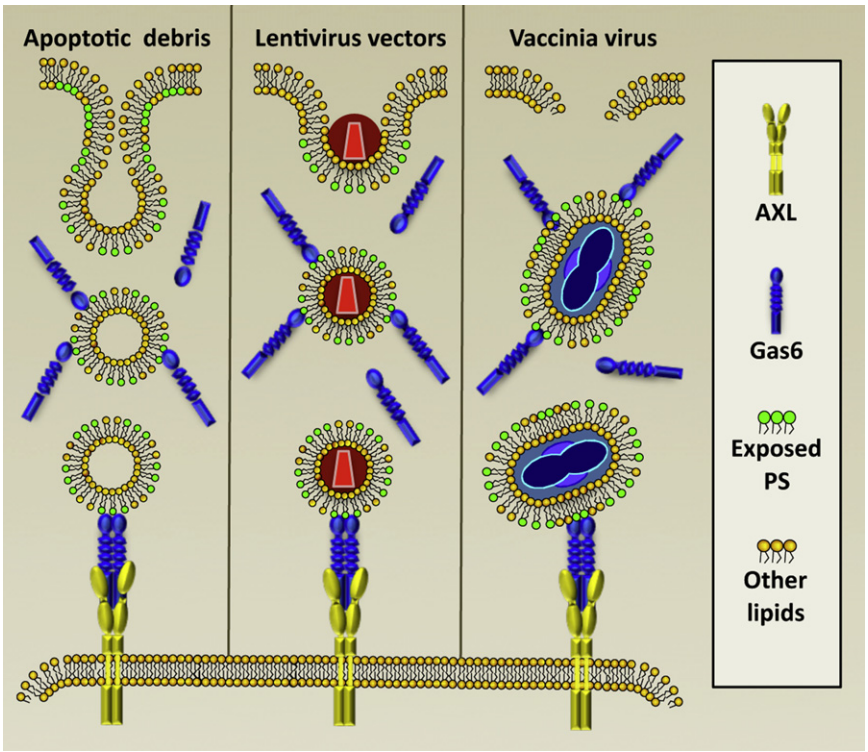

Figure 1. Model of Gas6/Axl-Mediated Virus Apoptotic Mimicry
During apoptosis, cells undergo several characteristic changes including membrane blebbing and redistribution of PS from the inner to the outer leaflet of the plasma membrane. The exposure of PS by these cells and their debris serves as the major "eat me" signal for their endocytic clearance. The TAM receptor Axl together with its cognate PS-bridging molecule, Gas6, can mediate the uptake of these particles. Gas6 serves as the link between the target PS and the receptor. Some viruses also accumulate PS in their envelope during the assembly process. Lentivirus vectors likely gain PS at the cell surface when they bud from infected cells. VACV acquires its PS-enriched membrane in the cytoplasm from an unknown source and exits the cells by lysis (MVs) or fusion (EVs). Lentiviruses and VACV in the guise of apoptotic debris can be recognized by soluble Gas6, which links the viral particles to the receptor Axl, resulting in enhanced infectivity.

that the surface arrangement or concentration of Gas6 on the target may dictate TAM receptor specificity.

To test the impact of Gas6/Axl-mediated enhancement on a replication competent virus that contains PS in its envelope, the authors used VACV. VACV is unique in that two types of infectious virions are produced during the life cycle: mature virions (MVs) and extracellular virions (EVs), which consist of an MV with an additional Golgi- or endosome-derived membrane. VACV MVs have been shown to require envelope PS for entry and infection and were proposed to use apoptotic mimicry for internalization (Mercer and Helenius, 2008). When MVs or EVs were treated with Gas6, enhancement of infection was moderate relative to that observed with the pseudotyped lentivirus vectors. As Gas6/Axl-mediated enhancement is most effective when lentivirus vector transduction is weak (Morizono et al., 2011), the authors speculate that the low level of enhancement seen with VACV may reflect the high specific infectivity of this virus. It is also possible that Gas6/Axl-mediated enhancement only works for a minor fraction of VACV virions, or that additional PS-binding factors and receptors are required for VACV infection. Importantly, the PS in the envelope of vaccinia MVs is not required for virus binding (Mercer and Helenius, 2008). This suggests that Gas6/Axl-mediated enhancement of VACV infection occurs at the level of cellular signaling or virus internalization. Additional studies will be required to determine the step of VACV entry facilitated by Gas6 and Axl.

The authors found that Gas6-mediated enhancement was greater for EVs than MVs, and that EV infection was enhanced on cells overexpressing Axl. However, the outermost EV membrane is fragile. EV purification can result in the disruption of this membrane and exposure of underlying MVs that contain a significant amount of envelope PS. As stated by the authors, a caveat to these results is that the impact of "contaminating" virion PS on Gas6-mediated enhancement of EV infection cannot be accounted for. Additional studies of Gas6/Axl-mediated enhancement of EV infection in the absence of contaminating PS-rich virions will be crucial. These findings highlight the fact that a thorough investigation of poxvirus binding and entry receptors is long overdue.

Collectively, these findings indicate that infectivity of pseudotyped lentivirus vectors and VACV is enhanced by Gas6, which serves to bridge PS in the virion envelope to the TAM receptor Axl (Figure 1). Thus, by mimicking apoptotic debris, these viruses subjugate the cells' clearance machinery to facilitate infection (Morizono et al., 2011). Like all interesting findings, the work by Morizono and colleagues raises many questions. First, why have these viruses subjugated the apoptotic clearance pathway? Evolutionarily, PS-mediated apoptotic clearance is ancient. It is highly conserved in *C. elegans*, *Drosophila*, and vertebrates and is indispensible for the function of these multicellular organisms. In tissues, dead and dying cells that expose PS are rapidly cleared, with PS exposure serving to suppress inflammatory responses. By using apoptotic mimicry, pathogens may assure their uptake, evade immune recognition, and at the same time stunt the host inflammatory response. It's tempting to speculate that the list of pathogens using apoptotic mimicry, already including protozoan parasites (Wanderley and Barcinski, 2010), will continue to grow.

Second, does the use of apoptotic mimicry by lentivirus vectors alter the endocytic mechanism they naturally use? Further studies will be needed to elucidate additional receptors, cellular signaling pathways, and endocytic mechanisms subjugated during PS-mediated uptake. Finally, and perhaps most important, does lentivirus vector and VACV apoptotic mimicry occur in vivo? For the lentivirus vectors, does this contribute to residual transduction and aberrant

cellular targeting? If so, how might this impact their therapeutic potential? It has already been demonstrated that antibodies directed against PS can neutralize lethal pichinde and cytomegalo virus infections in animals (Soares et al., 2008). For the poxviruses and other human pathogens using apoptotic mimicry (HIV, Ebola, etc.), can PS exposure be used as a potential antiviral target? Future efforts should be directed at exploiting pathogen PS exposure to this end.

The significance of viral proteins in the activation of host cell signaling and virus entry is well understood. The work by Morizono and colleagues will undoubtedly lead to future experimentation directed at understanding the role of virus lipids during these processes. Additionally, this study will likely stimulate further investigation of viral apoptotic mimicry mechanisms used by these and other viruses.

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Leishmania Parasites Act as a Trojan Horse that Paralyzes the Translation System of Host Macrophages

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GP63 is an abundant GPI-anchored surface metalloprotease of *Leishmania*. Jaramillo et al. (2011) show that GP63 manipulates the translation system of host macrophages by cleaving mTOR, which leads to 4E-BP1 dephosphorylation. This study pioneers the observation that *Leishmania* parasites metabolically paralyze their host cells using an elegant translation shutoff mechanism.

Leishmania parasites are the causative agents of a broad spectrum of diseases that affect millions of people across the Old and New Worlds and inflict morbidity and mortality when untreated. The parasites lead a complex life cycle, in which the flagellated promastigote form resides in the alimentary canal of female sand flies. During the blood meal, the parasites are transmitted into the circulation system of a mammal, where they are phagocytized by macrophages and subsequently transform into the nonmotile amastigote form. The obligatory intracellular amastigotes reside within phagolysosomal vacuoles and adopt sophisticated mechanisms that enable them to avoid the hostile defense system of their host organism. The interplay between the parasite and its host is a complex process, in which the paramount interest of the parasite is to restrict the immune and microbicidal activities of the macrophage, while keeping it alive as a nutritional source.

Leishmania parasites encode a highly abundant surface protein, GP63 (Bordier, 1987), which is associated with parasite virulence (McGwire et al., 2003). Due to its high abundance and its key role in the progression of the disease, GP63 has been a major target for efforts to develop a vaccine against *Leishmania.* GP63 is anchored to the cell membrane by a GPI-anchor and functions as a secreted zinc metalloprotease. It was recently reported to promote the cleavage-dependent activation of macrophage protein tyrosine phosphatases, as part of a refined mechanism that downregulates host cell functions and inhibits host defense systems (Contreras et al., 2010; Gomez et al., 2009).

In this issue, Jaramillo et al. unveil an interesting new role for the parasite virulence factor GP63 on the translation machinery of the infected macrophage (Jaramillo et al., 2011). The authors show