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Localization to detergent-resistant membranes and HIV-1 core entry inhibition correlate with HIV-1 restriction by SERINC5

Bianca Schulte[a], Anastasia Selyutina[a], Silvana Opp[a], Alon Herschhorn[b], Joseph G. Sodroski[b], Massimo Pizzato[c], Felipe Diaz-Griffero[a,*]

[a] Department of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, NY 10461, USA
[b] Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA 02215, USA
[c] University of Trento, Centre for Integrative Biology, 38123 Trento, Italy

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ABSTRACT

SERINC5(S5) is a multi-span transmembrane protein that potently blocks the infectivity of HIV-1 produced by human T-cells. The ability of S5 to restrict infectivity correlates with its presence in the virion, but the exact mechanism by which S5 restricts HIV-1 is unknown. Here we tested whether the core from HIV-1 virions containing S5 is delivered to the cytoplasm. Using the "fate of the capsid" assay, we demonstrated that the viral core of S5-restricted HIV-1 does not reach the cytoplasm of target cells, suggesting a block in the delivery of the core to the cytoplasm. In agreement with evidence suggesting that the viral determinants for S5 restriction map to the envelope of HIV-1, we observed that S5 induces conformational changes to the HIV-1 envelope. Further, we demonstrated that S5 localizes to detergent-resistant membranes (DRMs), as has been shown previously for the HIV-1 envelope in producer cells. In order to identify the determinants of S5 restriction, we explored the ability of all human SERINC proteins to restrict HIV-1. In contrast to human S5, we observed that human SERINC2(S2) did not restrict HIV-1, and was inefficiently incorporated into HIV-1 virions when compared to S5. Experiments using S5-S2 chimeric proteins revealed two functional domains for restriction: one necessary for S5 incorporation into virions, which does not seem to be necessary for restriction, and a second one necessary to change the HIV-1 envelope conformation, localize to DRMs, and block infection.

1. Introduction

Nef is a lentiviral accessory protein essential for viral replication and induction of AIDS-like symptoms in Rhesus macaques by a pathogenic SIV strain (Kestler et al., 1991). In 1994, Chowers and colleagues reported that primate lentiviral accessory protein Nef can enhance the infectivity of HIV-1 virions produced in human T cells (Chowers et al., 1994). Their experiments demonstrated that Nef increases HIV-1 replication in human T-cells. Similar results were reported subsequently by other investigators (Aiken and Trono, 1995; Schwartz et al., 1995). In 2015, two groups reported independently that the human proteins SERINC3 (S3) and SERINC5 (S5) are the HIV-1 restriction factors switched off by Nef (Rosa et al., 2015; Usami et al., 2015). S3 and S5 are members of the serine incorporator (SERINC) family of proteins, which is highly conserved among eukaryotes (Inuzuka et al., 2005).

Mechanistic studies have led to the following findings regarding the ability of S3 and S5 to restrict HIV-1 (Rosa et al., 2015; Usami et al., 2015): a) S3 and S5 are incorporated into the HIV-1 viral particle, which correlates with blockage of infectivity, and b) the ability of Nef to overcome restriction correlates with decreased incorporation of SERINC proteins into the virus. Although both reports suggested that there is a virus-cell fusion defect, Rosa and colleagues noted that the fusion pores between viruses and cells are formed (Rosa et al., 2015).

Early studies suggested that the envelope protein of HIV-1 is the determinant for the Nef-sensitive HIV-1 restriction imposed by T cells (Lai et al., 2011; Usami and Gottlinger, 2013). In agreement with these findings, recent evidence also linked the HIV-1 envelope to the restriction observed by S5 (Beitari et al., 2017; Sood et al., 2017). Altogether, these experiments suggested that S5 might be changing the conformation of the envelope through direct interaction or by changing the lipid composition of the membrane. So far no evidence has shown an interaction between S5 and the envelope of HIV-1. Although it has been suggested that expression of SERINC proteins increases phosphatidylserine synthesis in bacteria (Inuzuka et al., 2005), recent evidence showed that S5 does not alter the lipid composition of the HIV-1 virion (Trautz et al., 2017). This showed that much is left to be investigated

* Correspondence to: Felipe Diaz-Griffero, Department of Microbiology and Immunology, Albert Einstein College of Medicine, 1301 Morris Park – Price Center 501, New York, NY 10461, USA.
E-mail address: Felipe.Diaz-Griffero@einstein.yu.edu (F. Diaz-Griffero).

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regarding the mechanism used by S5 to block HIV-1. Although primate evolutionary studies showed that there are no notable sequence signatures for positive selection in S5 and S3 (Murrell et al., 2016), studies have shown that the Nef-mediated SERINC5 antagonism may determine the ability of primate lentiviruses to spread within natural hosts (Heigele et al., 2016).

Previous investigations showed that S5-containing HIV-1 particles do not undergo reverse transcription, suggesting that S5-containing HIV-1 viruses might not be able to deliver the viral core into the cytoplasm of target cells; therefore, we investigated whether the core of S5-containing HIV-1 is actually released into the cytoplasm of target cells. Using the "fate of the capsid" assay, we found that the HIV-1 core from S5-containing particles is not released into the cytoplasm of target cells, implying a defect in cytoplasmic delivery of the HIV-1 core. The viral determinants for S5 restriction have been mapped to the HIV-1 envelope, suggesting that S5 is critically affecting the function of the HIV-1 envelope. The presence of S5 in the viral particle was found to induce conformational changes in the HIV-1 envelope that correlated with restriction. Consistently with this, it was found that S5 localizes to detergent-resistant membranes (DRMs), as has been shown for the HIV-1 envelope in producer cells, suggesting that S5 and HIV-1 envelope co-localization in DRMs is important for restriction. To define in greater detail the determinants for this restriction, we sought to identify SERINC proteins that do *not* restrict HIV-1, and for this purpose, evaluated the ability of all human SERINC proteins to restrict HIV-1. We observed that human S2 did not restrict HIV-1, and was incorporated only inefficiently into HIV-1 when compared to S5. These results identified S2 as a suitable protein with which to construct chimeras that might help identify determinants for restriction. Gratifyingly, S5-S2 chimeric proteins revealed two important domains for restriction: one necessary for incorporation into viral particles, and a second domain necessary to change the HIV-1 envelope conformation, localize to DRMs, and block HIV-1 infection.

2. Results

2.1. Ability of human SERINC proteins to restrict HIV-1 infection

In order to begin our investigations on the mechanism by which S5 blocks HIV-1, we sought to find human SERINCs that differentially restrict HIV-1. The simultaneous study of the five human SERINC proteins will help defining the requirements for restriction. For this purpose, we tested the ability of all human SERINC proteins to restrict HIV-1 (Fig. 1). We challenged TZM-bl GFP-reporter cells with HIV-1_{SF162} particles produced in the presence of increasing concentrations of the indicated SERINC proteins (Fig. 1). At 48 h post-challenge, infection was determined by measuring the percentage of GFP-positive cells, and the results were used to calculate fold-restriction. At the same time, producer cells were analyzed for expression of SERINC proteins and GAPDH using anti-FLAG and anti-GAPDH antibodies, respectively. Similarly, SERINCs and p24 expression was analyzed in partially purified virions (using a 20% sucrose cushion). Detection of SERINCs required the use of a modified Western blot protocol described in Methods.

The use of increasing concentrations of S5 tagged with a FLAG epitope (S5-FLAG) revealed two blocks for HIV-1 infection (Fig. 1A): **1)** an HIV-1 infectivity block of ~10–40 fold was observed in released virions when low levels of S5-FLAG were detectable in producer cells (note: block did not affect viral production), and **2)** an artifactual block of HIV-1 infectivity of > 40 fold observed when high levels of S5-FLAG were expressed in virus- producing cells, which affected virus production and/or release as measured by blotting for p24. These results indicated that high S5 expression inhibits virion production, causing the latter to be an overexpression artifact; as shown in producer cells there was a decrease in Gag expression and maturation in producer cells when high levels of S5 were used (Fig. 1A). Therefore, all experiments in this manuscript will assess HIV-1 restriction using S5 levels that do not affect viral particle release, as restriction in endogenous T-cells was originally described (Chowers et al., 1994). Of note with regard to these experiments, S5-FLAG was shown to incorporate into HIV-1 virions (Fig. 1A). As shown in Fig. 1A, a black arrow points to the experiments where the levels of SERINC expression did not affect virus production as measured by p24, which also indicates the restriction fold we are considering for S5. This type of titration assays will be used through out this work to understand restriction of the different SERINC variants.

Similarly, the ability of different concentrations of S2 tagged with a FLAG epitope (S2-FLAG) to block the infectivity of released HIV-1 particles was tested. As shown in Fig. 1B, expression of S2 at different levels in producer cells did not negatively affect virion production or infectivity of released virions, which is in agreement with recent findings (Sood et al., 2017). Instead, we observed that S2 expression in producer cells was observed to enhance the infectivity of released HIV-1 by as much as two-fold. Our results suggested that S2 incorporates less efficiently into HIV-1 when compared to S5 (Fig. 1B). Because of its inability to block the infectivity of released viruses, S2 serves as a useful control for experiments designed to elucidate how S5 affects HIV-1 infection.

Next we tested restriction of HIV-1 infection by S4, the closest homologue to S5, using increasing amounts of S4 (Fig. 1C and Fig. S1). Interestingly, expression of S4 tagged with a FLAG epitope (S4-FLAG) potently blocked infectivity of released viruses (Fig. 1C). Similarly to S5, the use of high S4 expression resulted in a decrease of Gag expression and maturation (Fig. S1). As a control we included S2-FLAG, which does not restrict the infectivity of released virions. These results showed that at similar levels of total expression S4 blocks infectivity of HIV-1 as potently as S5 (Fig. S1).

Expression of S3 tagged with a FLAG epitope (S3-FLAG) in producer cells diminished the infectivity of released HIV-1 virions two- to three-fold (Fig. 1C), demonstrating that S3 is a less potent restriction factor than S5, as shown earlier (Rosa et al., 2015; Usami et al., 2015).

Expression of S1 tagged with a FLAG epitope (S1-FLAG) in producer cells blocked the infectivity of released HIV-1 virions only minimally (Fig. 1C), indicating that S1 has limited or no activity against HIV-1.

2.2. S5 topology and contribution of the different loops to HIV-1 restriction

To understand the contribution of the S5 loops to restriction, we first sought to understand the membrane topology of S5. Members of the SERINC family were originally described as membrane proteins containing 10–11 transmembrane domains (Inuzuka et al., 2005). The model simulated by TOPCONS (http://topcons.cbr.su.se) predicts that S5 contains ten transmembrane domains (TM), five extracellular loops, and four intracellular loops (L) (Fig. 2A). Based on this model, we explored the membrane topology of S5 using flow cytometry by testing surface (fixed) and total (fixed & permeabilized) expression of S5 variants. S5 proteins containing a FLAG epitope peptide inserted into each of the nine loops (L1-9) were used. As shown in Fig. 2A, S5 tagged with a C-terminal FLAG epitope showed poor surface detection (fixed) when compared to total expression (fixed & permeabilized), suggesting that the C-terminus of S5 resides within the intracellular compartment. By contrast S5 containing a FLAG epitope in L7 (S5-L7-FLAG) or L9 (S5-L9-FLAG) showed similar surface detection versus total expression, suggesting that L7 and L9 reside in the extracellular compartment. In a similar manner, we showed that L2, L4, L8 and the C-terminal tail of S5 face toward the intracellular compartment (Fig. 2A). The remaining constructs were poorly expressed, preventing us from drawing firm conclusions. Overall, however, our observations were in agreement with the notion that L7 and L9 are facing out toward the extracellular compartment, whereas the L2, L4, L8 and the C-terminal region of S5 face toward the intracellular compartment. To corroborate these results, immunofluorescence staining experiments were performed using anti-FLAG antibodies. S5-L7-FLAG and S5-L9-FLAG showed a similar staining pattern when compared to wild-type S5 (Fig. 2B). Taken