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Primate lentiviruses are differentially inhibited by interferon-induced transmembrane proteins

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ABSTRACT

Interferon-induced transmembrane (IFITM) proteins inhibit the entry of a large number of viruses. Not surprisingly, many viruses are refractory to this inhibition. In this study, we report that different strains of HIV and SIV are inhibited by human IFITM proteins to various degrees, with SIV of African green monkeys (SIV_{AGM}) being mostly restricted by human IFITM2. Interestingly, SIV_{AGM} is as much inhibited by human IFITM2 as by IFITM3 of its own host African green monkeys. Our data further demonstrate that the entry of SIV_{AGM} is impaired by human IFITM2 and that this inhibition is overcome by the cholesterol-binding compound amphotericin B that also overcomes IFITM inhibition of influenza A viruses. These results suggest that IFITM proteins exploit similar mechanisms to inhibit the entry of both pH-independent primate lentiviruses and the pH-dependent influenza A viruses.

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Introduction

Interferon-induced transmembrane (IFITM) proteins inhibit a wide range of viruses (reviewed in (Diamond and Farzan, 2013; Perreira et al., 2013)). Humans have five IFITMs including IFITM1, 2, 3, 5 and 10 among which IFITM1, 2 and 3 exhibit antiviral activities (Hickford et al., 2012). These three IFITMs are ubiquitously expressed in different tissues, and respond to type I interferon stimulation. IFITM5 is strictly expressed in osteoblasts and has a role in bone mineralization (Moffatt et al., 2008). The function of IFITM10 remains unknown. Many important human pathogenic viruses are sensitive to IFITM restriction. These include influenza A virus, flaviviruses, Ebola virus, SARS coronavirus, Rift Valley fever virus, reovirus, human immunodeficiency virus type 1 (HIV-1), etc. (Anafu et al., 2013; Brass et al., 2009; Huang et al., 2011; Jiang et al., 2010; Lu et al., 2011; Mudhasani et al., 2013). The importance of IFITM proteins in host antiviral defense is demonstrated by the high mortality of *ifitm*3-knockout mice infected with influenza A virus and by the possible association of an SNP in the human *ifitm*3 gene with the disease severity caused by influenza virus infection (Bailey et al., 2012; Everitt et al., 2012; Wakim et al., 2013; Zhang et al., 2013).

IFITMs are small transmembrane proteins containing 120 to 135 amino acids (Siegrist et al., 2011). IFITM2 and 3 share higher homology as compared to IFITM1, which has a relatively shorter N-terminal region and a longer C-terminal region. IFITM proteins have two predicted transmembrane (TM) domains. However, recent data suggest that only the C-terminal TM domain of IFITM3 crosses the membrane, whereas the N-terminal one serves as an intramembrane domain (IMD) (Bailey et al., 2013; Jia et al., 2012; Yount et al., 2012). This IMD likely associates with the cytoplasmic leaflet of the lipid bilayer with the help of palmitoylated cysteine residues (Yount et al., 2012). This type of membrane topology allows the cytoplasmic exposure of a large portion of IFITM sequences that may interact with cellular factors and machineries that collectively modulate the functions of IFITMs. One example is the 20-YEML-23 motif of IFITM3 that interacts with the adaptor protein AP-2 and regulates IFITM3 endocytosis from the plasma membrane en route to late endosomes (Chesarino et al., 2014; Jia et al., 2012). The K24 residue of IFITM3 is a major site of ubiquitination. This modification affects IFITM3 subcellular localization and antiviral activity (Yount et al., 2012).

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The IFITM proteins inhibit virus infection by impairing virus entry (Feeley et al., 2011). Two models of inhibition have been proposed. One model suggests that IFITM proteins interfere with membrane hemifusion (Li et al., 2013). This model is supported by the results that IFITM proteins suppressed cell membrane hemifusion that was created by low pH at cold temperature. This inhibition was rescued by oleic acid that promotes membrane hemifusion (Li et al., 2013). The second model proposes that IFITM proteins impede the formation of viral fusion pore (Desai et al., 2014). In support of this latter model, no effect was detected on lipid mixing between viral membrane and endosomal membrane upon IFITM3 overexpression. Yet, the release of viral genome into the cytoplasm was blocked by IFITM3 (Desai et al., 2014). Both models are consistent with the notion that IFITM proteins can modulate the biophysical property of lipid bilayer, such as membrane fluidity and curvature, through mechanisms that possibly involve the interaction of IFITMs with VAPA and the disruption of intracellular cholesterol homeostasis (Amini-Bavil-Olyaee et al., 2013). In support of this mechanism, a cholesterol-binding agent amphotericin B overcomes the inhibition of influenza A virus infection by IFITM3 (Lin et al., 2013).

Not all enveloped viruses are inhibited by IFITM proteins. Examples are lymphocytic choriomeningitis virus (LCMV), Lassa virus (LASV), Machupo virus (MACH), human papillomavirus, cytomegalovirus and adenovirus that are all resistant to IFITMs (Brass et al., 2009; Warren et al., 2014). Among retroviruses, murine leukemia virus (MLV) is relatively refractory to IFITMs, the HIV-1 strain BH10 is inhibited, whereas another HIV-1 strain IIIB exhibits resistance (Brass et al., 2009; Lu et al., 2011). In this study, we examined a panel of HIV and SIV strains for their sensitivity to IFITM inhibition. The results revealed various degrees of inhibitions ranging from no inhibition for HIV-$1_{A/G}$ to 10-fold inhibition for SIV of African green monkeys.

Results

IFITM proteins inhibit primate lentiviruses

In order to evaluate the susceptibility of different primate lentiviruses to inhibition by IFITM1, 2 and 3, we selected the following viruses for study, including three HIV-1 strains (the laboratory adapted strain NL4-3, primary isolate YU-2 and the circulating recombinant form A/G), one HIV-2 strain (HIV-2_{Rod}), five SIV strains from chimpanzees ($SIV_{CPZ1.9}$), African green monkeys *Chlorocebus sabaeus* ($SIV_{AGM\text{-}sab}$) and *Chlorocebus tantalus* ($SIV_{AGM\text{-}tan}$), rhesus macaques ($SIV_{MAC\text{-}1A11}$) and sooty mangabeys (SIV_{SMM}). Since these viruses either use CXCR4 or CCR5 as the co-receptor, we chose to infect the HIV indicator cell line TZM-bl that expresses both CXCR4 and CCR5 and are thus susceptible to infection by all these viruses. We first transduced TZM-bl cells with retroviral vectors expressing human IFITM1, 2 or 3 and selected the stably transduced cell lines with puromycin. Ectopic expression of IFITM1, 2 and 3 was confirmed by western blotting (Fig. 1A). We then challenged these TZM-bl cell lines with different doses of HIV or SIV. Virus infection was monitored by measuring luciferase activity that was expressed under the control of HIV-1 LTR promoter in TZM-bl cells. The data report the effect of IFITM proteins on the early phase of HIV/SIV infection until viral Tat protein is produced. Fig. 1B shows the luciferase activities of one representative infection experiment that was performed with different doses of viruses. The averages of three independent experiments are summarized in Fig. 1C. The results showed that $SIV_{AGM\text{-}tan}$ was inhibited the most, whereas infection of HIV-1, $SIV_{CPZ1.9}$ and SIV_{MAC} were not profoundly affected by the three human IFITM proteins. On the basis of the degrees of inhibition, these primate lentiviruses are ranked as $SIV_{AGM\text{-}tan} > SIV_{AGM\text{-}sab}$, SIV_{SMM}, HIV-2_{Rod} > HIV-$1_{NL4\text{-}3}$, HIV-$1_{YU\text{-}2}$, HIV-$1_{A/G} > SIV_{CPZ1.9}$ and SIV_{MAC}. The results also revealed that IFITM2 was the most inhibitory, followed by IFITM3 and IFITM1.

IFITM2 strongly diminishes the entry of SIV_{AGM}

Since IFITM proteins are known to inhibit virus entry (Feeley et al., 2011; Lu et al., 2011), we asked whether the strong inhibition of SIV_{AGM} by IFITM2 is a result of impaired virus entry. To this aim, we prepared the BlaM-Vpr-containing HIV and SIV particles, and used these virions to infect IFITM-expressing TZM-bl cells. The efficiency of virus entry was determined by measuring the cleavage of CCF2 by BlaM-Vpr that enters the cytoplasm together with viral cores. The results showed that the entry of HIV-$1_{NL4\text{-}3}$ and SIV_{MAC} into TZM-bl was marginally affected by IFITM1, 2 or 3 (Fig. 2). In contrast, the entry of $SIV_{AGM\text{-}tan}$ and $SIV_{AGM\text{-}sab}$, to a lesser extent SIV_{SMM}, was strongly impaired by IFITM2 and IFITM3 (Fig. 2). This similar reduction in the entry of $SIV_{AGM\text{-}tan}$ and $SIV_{AGM\text{-}sab}$ contrasts with a moderately stronger inhibition of $SIV_{AGM\text{-}tan}$ infection by IFITM2 and 3 as shown in Fig. 1. This difference suggests that $SIV_{AGM\text{-}tan}$ may be inhibited not only at the entry step, but also at a downstream step until viral Tat is produced, which is measured in the assays shown in Fig. 1.

We next asked whether the endogenous IFITM2 and 3 are able to inhibit the entry of SIV_{AGM}. We first used shRNA to knock down IFITM2 and 3 in TZM-bl cells (Fig. 3A). Both $SIV_{AGM\text{-}tan}$ and $SIV_{AGM\text{-}sab}$ showed significantly higher infection in the IFTIM2/3-knockdown cells (Fig. 3B). Since the BlaM-Vpr containing $SIV_{AGM\text{-}sab}$ particles generated much stronger signals in the entry assay than $SIV_{AGM\text{-}tan}$ (Fig. 2), we further measured the effect of IFITM2/3-knockdown on the entry of $SIV_{AGM\text{-}sab}$. We also treated TZM-bl cells with IFNα2b to increase the expression of endogenous IFITM2 and 3. The results showed that IFNα2b reduced the entry of $SIV_{AGM\text{-}sab}$ by 2-fold and this diminution was completely lost when IFITM2 and 3 were depleted with shRNA (Fig. 3C and D). When the endogenous IFITM2 and 3 were knocked down in a human T cell line called C8166 that constitutively express relatively high level of IFITM2, the entry of $SIV_{AGM\text{-}sab}$ increased by approximately 50% (Fig. 3E–G). IFNα2b treatment increased the expression of IFITM2 and 3, and results in a 40% reduction in $SIV_{AGM\text{-}sab}$ entry. Depletion of IFITM2 and 3 under IFNα2b treatment restored $SIV_{AGM\text{-}sab}$ entry to the control level (Fig. 3E and F). We also observed that shRNA3 depleted IFITM2 much more efficiently compared to shRNA1 and shRNA2 (Fig. 3E), which correlates with a moderately greater entry of $SIV_{AGM\text{-}sab}$ in shRNA3-transdued C8166 cells than in shRNA1- or shRNA2-transduced cells, albeit that this increase does not reach statistical significance (Fig. 3F and G). Together, these data indicate that endogenous IFITM2 and 3 inhibit the entry of $SIV_{AGM\text{-}sab}$.

Amphotericin B overcomes the inhibition of SIV_{AGM} by IFITM2 and 3

It has been reported that amphotericin B prevents IFITM3 from inhibiting influenza A virus through modulating membrane fluidity (Lin et al., 2013). We suspected that, if IFITM2 and 3 use the same mechanism to inhibit SIV_{AGM} and influenza A virus, then amphotericin B should also rescue the infection of SIV_{AGM} in IFITM2/3-expressing cells. Indeed, when amphotericin B was added with increasing doses, the infection of both $SIV_{AGM\text{-}sab}$ and $SIV_{AGM\text{-}tan}$ in IFITM2 or IFITM3-expressing TZM-bl cells were restored to the level of infection in control cells (Fig. 4A and B). HIV and SIV are known as pH-independent viruses (McClure et al., 1988). Yet, the high sensitivity of SIV_{AGM} to IFITM2 and 3 inhibition raises the possibility that this SIV may have become pH-dependent similar to the influenza A virus. Contrary to this speculation, SIV_{AGM} showed resistance to the treatment of chloroquine or bafilomycin A1 both of which neutralize

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