Species tropism of HIV-1 modulated by viral accessory proteins

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INTRODUCTION

Human immunodeficiency virus type 1 (HIV-1) is strictly adapted to humans, and does not replicate in macaque monkeys routinely used for experimental infections. This specially narrow host range (species tropism) has impeded much the progress of HIV-1 acquired immunodeficiency syndrome (AIDS) basic research. Extensive studies on the underlying mechanism have revealed that Vif, one of viral accessory proteins, is critical for the HIV-1 species tropism in addition to Gag-capid protein. Another auxiliary protein Vpu also has been demonstrated to affect this HIV-1 property. In this review, we focus on functional interactions of these HIV-1 proteins and species specific-restriction factors. In addition, we describe an evolutionary viewpoint that is relevant to the species tropism of HIV-1 controlled by the accessory proteins.

Keywords: HIV-1, species tropism, accessory protein, Vif, Vpu

VIRAL AND CELLULAR DETERMINANTS FOR HIV-1 SPECIES TROPISM

Our early studies have already suggested the possible viral determinants and viral replication stage involved in the HIV-1 species tropism described above (Shibata et al., 1991, 1995; Shibata and Adachi, 1992). By the use of numerous chimeric molecular clones between HIV-1 and dual-tropic (tropic for human and monkey cells) SIVmac (isolated from the macaque monkey), we have claimed, in essence, together with a work on the cyclophilin A (CypA), that Gag-capid (CA) and a viral protein(s) encoded by the central genomic region of HIV-1 are the determinants. We also have showed that HIV-1 is replication-incompetent in monkey cells because a certain replication step(s) before/during reverse transcription, other than the viral entry into cells, does not proceed normally. Subsequent extensive studies by us and others have clearly indicated that the interactions of Gag-CA/CypA, Gag-CA/tripartite motif (TRIM) proteins, and Vif/apolipoprotein B mRNA-editing enzyme-catalytic (APOBEC) proteins are major determinants for the HIV-1 species tropism (Nomaguchi et al., 2008, 2011; Nakayama and Shioda, 2012; Sakuma and Takeuchi, 2012) as summarized in Table 1. Gag-CA, CypA, and TRIM proteins have been described in detail in two articles in the Research Topic of this journal (Nakayama and Shioda, 2012; Sakuma and Takeuchi, 2012).

The biological and molecular biological bases for species tropism of HIV-1 should reside in the above outlined evolutionary processes. In particular, the so-called accessory proteins encoded by extra genes are important. Each virus group has a unique set of the accessory proteins in terms of their combinations and of their activities. Therefore, studies on viral accessory proteins are also meaningful for understanding viral evolution by cross-species transmission.

Human immunodeficiency virus type 1 (HIV-1) is tropic and pathogenic only for humans, and does not replicate in macaque monkeys routinely used for experimental infections. This specially narrow host range (species tropism) has impeded much the progress of HIV-1 acquired immunodeficiency syndrome (AIDS) basic research. Extensive studies on the underlying mechanism have revealed that Vif, one of viral accessory proteins, is critical for the HIV-1 species tropism in addition to Gag-capid protein. Another auxiliary protein Vpu also has been demonstrated to affect this HIV-1 property. In this review, we focus on functional interactions of these HIV-1 proteins and species specific-restriction factors. In addition, we describe an evolutionary viewpoint that is relevant to the species tropism of HIV-1 controlled by the accessory proteins.
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**FIGURE 1** Genome organization of primate immunodeficiency viruses. Various proviral genomes are schematically shown. As indicated by colored boxes, the vpr and vpu genes of SIVcpz/HIV-1 came from those of SIVrcm and SIVmon/mus/gsn, respectively. Also, the vif genes of SIVcpz/HIV-1 originated from that of SIVrcm. In addition, as shown by colored boxes, HIV-1 nef gene is similar to but distinct from SIVcpz nef gene. HIV-1 nef gene is different from those of SIVmon/mus/gsn, SIVrcm, and HIV-2 as indicated. For virus designations, see text.

**Table 1** Major viral and cellular determinants for HIV-1 species tropism.

| Virus | Cell | Viral replication step affected |
|-------|------|---------------------------------|
| Gag-CA | CypA | Uncoating (early phase) |
| Gag-CA | TRIM5α | Uncoating (early phase) |
| Gag-CA | TRIMCyp | Reverse transcription (early phase) |
| Vif | APOBEC3G | Reverse transcription (early phase) |
| Vpu | Tetherin/BST-2 | Virion release (late phase) |

For details, see references (Yokoyama and Shioda, 2012; Sakuma and Takeuchi, 2012) for Gag-CA, and Figures 3 and 4 for Vif/Vpu.

**ACCESSORY PROTEINS OF PRIMATE IMMUNODEFICIENCY VIRUSES**

All primate immunodeficiency viruses encode a number of extra proteins (Vif, Vpx, Vpr, Vpu, and Nef) in addition to regulatory (Tat and Rev) and structural (Gag, Pol, and Env) proteins (Figure 1). Structural proteins are common to all retroviruses, but the regulatory and accessory proteins are unique to the complex primate lentiviruses and not found in the other simple mammalian retroviruses. Regulatory Tat and Rev proteins are trans-activators for transcription and for the expression of late viral proteins, respectively. While the regulatory and structural proteins are essential for viral replication, the extra proteins, usually generically called "accessory," are dispensable under certain circumstances. However, in some cells, some of them are essential and the others are quite critical/important for optimal viral replication as illustrated for ΔVif and ΔVpu viruses (viruses that lack Vif or Vpu) in Figure 2. Another point to be mentioned here is relating to Vpr/Vpx proteins. Although Vpr and Vpx are genetically very similar (Khamsri et al., 2006), some primate immunodeficiency viruses bear two of them as described above (Fujita et al., 2010). Furthermore, the other viruses have Vpr only. What about the functional relationship of the two proteins? At present, the function of Vpr/Vpx is least well understood relative to that of the other accessory proteins (Malim and Emerman, 2008; Fujita et al., 2010). Table 2 summarizes the important information regarding these accessory proteins so
FIGURE 2 | A schema of replication kinetics by HIV-1 wild-type and mutant viruses. Viral growth properties in cells are illustrated based on numerous infection experiments in our laboratory. WT, wild-type.

FIGURE 3 | HIV-1 replication and APOBEC3G. On the basis of results reported so far, the action mechanism of Vif is depicted. Replication process for wild-type (WT) and ΔVif mutant viruses are schematically shown on the basis of previously reported review articles (Holmes et al., 2007; Huthoff and Towers, 2008; Strebel et al., 2009). A3G, APOBEC3G; IN, viral integrase protein.

Table 2 | Accessory proteins of primate immunodeficiency viruses.

| Viral Proteins | Major functions for viral replication reported so far |
|---------------|----------------------------------------------------|
| Vif           | Neutralize APOBEC3G/F. Essential for viral replication in natural target cells. |
| Vpx           | Degrade SAMHD1/APOBEC3A. Critical for viral replication in natural target cells. |
| Vpr           | Important for viral replication in macrophages (HIV-1). |
| Vpu           | Down-regulate Tetherin/BST2. Important for viral replication in CD4-positive cells. |
| Nef           | Down-regulate cell surface molecules (CD4, MHC-I etc.). |

far reported. In total, it is fairly reasonable to believe that the accessory proteins are regulators to optimize viral replication and persistence in vivo thereby enhancing viral transmission between individuals.
Vif AND Vpu PROTEINS

Vif protein is essential for viral replication in natural target cells such as CD4-positive lymphocytes and macrophages. Recent identification of its cellular object for attack (Sheehy et al., 2002) has clearly revealed the biological and biochemical bases for the growth property of ΔVif virus in natural target cells. This finding (identification of a family of APOBEC3 proteins, cellular cytidine deaminases, as potent inhibitors of HIV-1 replication in primary cells) has also contributed much to establish the concept of “the restriction factor” to well understand virus-cell interaction (Malim and Emerman, 2008; Sato et al., 2012).

Of the APOBEC3 family proteins, APOBEC3G and APOBEC3F (Kitamura et al., 2011) strongly inhibit viral replication in the absence of Vif (Figure 3). Although HIV-1 Vif can abrogate the activities of human APOBEC3, it cannot do so against monkey APOBEC3. In contrast, SIVmac Vif can neutralize the antiviral activity of APOBEC3 of both origins. Finally, it has been demonstrated that Vif and APOBEC3 are the major determinants for the HIV-1 species tropism by constructing macaque-tropic HIV-1 (HIV-1mt) and monitoring the HIV-1mt growth property in various genetic contexts of macaques (Hatzioannou et al., 2006, 2009; Kamada et al., 2006; Igarashi et al., 2007; Thippeshappa et al., 2011).

Vpu protein, unique to viruses of the HIV-1 group (Figure 1), modulates viral replication in human CD4-positive cell lines and primary cells. Mutant HIV-1 without Vpu (ΔVpu virus) grows poorly relative to wild-type virus. Recently, a cellular protein named Tetherin (also called BST-2) has been identified as a restriction factor against HIV-1 and is antagonized by Vpu (Neil et al., 2008; Van Damme et al., 2008). Vpu down-regulates the Tetherin from cell surface, and thereby promotes extracellular production of progeny virions (Malim and Emerman, 2008; Arias et al., 2011; Sato et al., 2012). The baseline mechanism for this action of Vpu is well studied as shown in Figure 4. Here, it must be attentive that the anti-Tetherin activity of Vpu is host species-specific as observed for Vif. HIV-1 Vpu acts against human but not (or poorly) macaque Tetherins (Sauter et al., 2009, 2010). Although the biological effect of Vpu is much milder than that of Vif as judged by the growth kinetics of mutant viruses (Figure 2), Vpu may be critical for interspecies transmission.
Vpx and Vpr proteins are necessary for efficient viral replication and Tetherin/BST-2, respectively, and represent viral determinants for the host range of HIV-1 (Tables 1 and 2). It is intriguing to note that these factors would have shaped HIV-1 and made it unique among various primate immunodeficiency viruses (Figure 1).

Vpx AND Vpr PROTEINS

Vpx and Vpr proteins are necessary for efficient viral replication (Malim and Emerman, 2008; Fujita et al., 2010). In macrophages, ΔVpx replication is not detectable and this defect has been shown to be present at post-entry and before/during the reverse transcription process (Fujita et al., 2008, 2010; Srivastava et al., 2008). Also in some lymphocyte cell lines and in primary lymphocytes, Vpx protein is critical for viral replication (Ueno et al., 2003; Fujita et al., 2008). Because ΔVpr virus is somewhat replication-defective in some cells (for both HIV-1 and HIV-2), it is not unreasonable to assume that Vpr may play a role in the viral growth cycle. As such, Vpx and Vpr are important in vivo for viral replication and finally for viral pathogenicity (Fujita et al., 2010).

Very recently, SAMHD1 and APOBEC3A have been reported to be the myeloid cell-specific restriction factors against HIV-1 counteracted by Vpx (Berger et al., 2011; Hercka et al., 2011; Lagayette et al., 2011). Whether these proteins are associated with the HIV-1 species tropism described in this review article, and whether they can explain the in vitro and in vivo situation of HIV-2/SIVmac mutant viruses mentioned above remain to be determined (Fujita et al., 2010; Nomaguchi et al., 2011).

CONCLUSION

In this review, we have described the major determinants for the species tropism of HIV-1. Structural Gag–CA and accessory Vif and Vpx proteins are clearly involved in this host range of HIV-1 as viral factors (Table 1). Cellular proteins that interact with these and contribute to this tropism are definitely the restriction factors (Table 1). In total, interplays between the viral and cellular responsible factors decide this unique and limited tropism of HIV-1. Whether there are other factors that affect the HIV-1 species tropism is awaiting further investigations. In this regard, the biology of Vpx deserves attention. Because Vpx is present in SIVmac but not in HIV-1 (Figure 1), it may inactivate a cellular anti-viral protein(s) which is not recognized by HIV-1 proteins.

In both basic and applicable points of view, the narrow host range of HIV-1 is burdensome obstacle to overcome. Assuming that HIV-1mt can grow and cause disease similarly with SIVmac in macaques, we would be able to better perform model studies to precisely analyze viral replication and pathogenicity in vivo, and to establish the effective anti-HIV-1/AIDS strategies. To the best of our knowledge, there are no such HIV-1mt clones so far (Hatzioannou et al., 2006, 2009; Kamada et al., 2006; Igarashi et al., 2007; Kuroishi et al., 2009; Saito et al., 2011; Thippeshappa et al., 2011). We may further improve the ability of HIV-1mt by today’s powerful methodology if we knew all the cellular determinants for the species tropism of HIV-1. Studies in this direction are in progress in our laboratory.

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