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Macaque-Tropic HIV-1 Derivatives: A Novel Experimental Approach to Understand Viral Replication and Evolution In Vivo

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1. Introduction

The use of animal models in the study of human diseases is obviously important. Fundamental properties of the disease can be investigated analytically and thoroughly by this approach, contributing much to the progress of basic science as well as clinical medicine (Nomaguchi & Adachi, 2010). Researchers in various specialties, therefore, have made every effort to establish animal models for human diseases including those caused by infectious agents. Acquired immunodeficiency syndrome (AIDS) of humans has long been one of the major targets for the model study in appropriate animals. However, human immunodeficiency virus type 1 (HIV-1) itself, the predominant causative virus of human AIDS, can not be used due to its very narrow host range. Because HIV-1 has adapted itself dexterously from the ancestral virus to replicate, persist and spread strictly in humans, it is very unique among various primate immunodeficiency viruses and no good counterparts are available in nature (Desrosiers, 2007; Kirchhoff, 2009; Sauter et al., 2009). Therefore, it can be concluded that practical and meaningful animal systems of non-alternative nature for HIV-1 study do not exist at all to date, although there are pre-existing animal models of some significance.

HIV-1 does not replicate in animal species except for chimpanzees and humans (Nomaguchi et al., 2008a). Animals frequently used for our experiments on virology, such as rodents and nonhuman primates, are not exceptions to this barrier. However, if we are to search for, develop and establish a fruitful animal model system for HIV-1 research, nonhuman primates are considered to be most suited, for HIV-1 is best fitted with humans and some apes. Ever since the discovery of HIV-1 (Barre-Sinoussi et al., 1983), many prominent researchers keen on understanding its biology and molecular biology have done investigations extensively to elucidate the bases underlying the species-specificity unique to HIV-1. These studies have highlighted the presence of potent anti-HIV-1 factors in nonhuman cells that efficiently restrict or even abolish the replication of HIV-1 and successfully raised an epoch-making notion of the intrinsic immunity (Andrew & Strebel, 2010; Arhel & Kirchhoff, 2010; Ayinde et al., 2010; Bergamaschi & Pancino, 2010; Douglas et al., 2010; Fujita et al., 2010; Huthoff & Towers, 2008; Kirchhoff, 2010; Luban, 2007; Malim & Emerman, 2008; Nakayama & Shioda, 2010; Nomaguchi et al., 2008a, 2008b; Planelles & Barker, 2010; Sauter et al., 2010; Strebel et al., 2009; Towers, 2007). Cellular factors shoulder

this intrinsic immunity known to date are cyclophilin A (CypA) (Franke et al., 1994; Thali et al., 1994), apolipoprotein B mRNA-editing enzyme-catalytic polypeptide-like 3G (APOBEC3G)/APOBEC3F (Sheehy et al., 2002), tripartite motif protein 5α (TRIM5α) (Stremlau et al., 2004), TRIMCyp (Nisole et al., 2004; Sayah et al., 2004), and tetherin (alternatively called BST-2) (Neil et al., 2008; Van Damme et al., 2008). Because HIV-1 can indeed counteract human orthologs of these restriction factors effectively, it is well anticipated that HIV-1 in turn can be genetically engineered to replicate efficiently in nonhuman primates such as macaques. Nonetheless, most likely due to the lack of extensive and appropriate biological studies, we are still forced to use macaque-derived simian immunodeficiency virus (SIVmac) or SIVmac chimeric with a small portion of HIV-1 (SHIV) as an input virus for in vivo model studies in macaques. SIVmac and SHIV are genetically and biologically distinct from HIV-1 in a number of critical points, albeit they are quite similar to HIV-1 in the genome organization and pathogenic potentials (Desrosiers, 2007; Freed & Martin, 2007). They might not be used for future model studies aimed at understanding the biology of HIV-1 as a highly replicable/mutable, persistent, and pathogenic virus. We must go behind the outward form to grasp the inner meaning of the phenomenon, i.e., the species-specificity.

On the collective basis of molecular and biochemical studies performed by us and others so far, we recently have constructed a series of HIV-1 derivative clones tropic for macaque cells and/or macaques (Hatcho et al., 2008; Igarashi et al., 2007; Kamada et al., 2006, 2009; Kuroishi et al., 2009; Nomaguchi et al., 2008a; Saito et al., 2011; Yamashita et al., 2008), and are currently further modifying them for in vivo studies (our unpublished results). The viruses we have generated carry a minimal sequence of SIVmac, and overcome at least some species barriers. Importantly, these viruses are regarded to be genetically HIV-1, since they have less than 10% SIVmac genetic content (Igarashi et al., 2007). While we firmly believe that HIV-1 derivative viruses already constructed in our laboratory are useful for a variety of studies on HIV-1 infection in individuals, further improvement of the viruses by deliberating the evolutional process of SIV/HIV would surely add more scientific significance to basic and applied research fields. Needless to say, our goal is to generate a macaque-tropic HIV-1 (HIV-1mt) that replicates efficiently and is pathogenic for macaques as a standard pathogenic SIVmac clone such as SIVmac239 (Kestler et al., 1990). Through construction and biochemical/biological characterization of the ideal HIV-1mt clone with ability to induce AIDS at least in some species of macaques, we would be able to clarify the detailed molecular mechanisms for the narrow host range (species-tropism) of HIV-1. Viral Gag-capsid (CA) and accessory proteins (Vif, Vpx, Vpr, Vpu and Nef) are targets for those studies as a matter of course. Moreover, by using this persistent and pathogenic HIV-1mt clone as a seed virus for macaque infection experiments, we can trace and analyze its mutation, adaptation, evolutional direction to generate viral quasi-species, and finally pathogenesis in the context of immunological interaction. In addition, we can evaluate and develop the anti-HIV-1 drugs/vaccines by this HIV-1mt/macaque system.

In this chapter, we first outline the early and current studies on HIV-1, SIVmac and SHIV to emphasize and address the unique characteristics of HIV-1 and scientific issues to resolve. We then describe viral and cellular factors that are responsible for or potentially associated with restriction of viral replication. We finally focus on our recent studies on the strategies to obtain HIV-1mt clones and on the biology/molecular biology of HIV-1mt clones. Main parts of this chapter consist of: (i) Overview of the biology and molecular biology of HIV-1, SIVmac and SHIV; (ii) Determinants for HIV-1 species-tropism; (iii) Generation and

characterization of various HIV-1mt clones. The primary mission of we basic virologists is to understand viral replication and viral pathogenesis in vivo by multilateral approaches (Nomaguchi & Adachi, 2010). We then take over our new important findings to functional parties in related fields, thus promoting further progress in virology.

2. Overview of the biology and molecular biology of HIV-1, SIVmac and SHIV

Numerous immunodeficiency viruses of distinct groups have been isolated from humans and a wide variety of African nonhuman primates (Desrosiers, 2007; Freed & Martin, 2007). These viruses infect the immune system of primates, kill cells that are critical for effective immune responses, and eventually cause AIDS in some hosts (Desrosiers, 2007; Kuritzkes & Walker, 2007). Soon after the discovery in 1983 (Barre-Sinoussi et al., 1983; Barre-Sinoussi, 2010; Montagnier, 2010), HIV-1 was demonstrated to belong to a lentiviral genus of the retrovirus family, and expected to exhibit the properties characteristic of the family (Goff, 2007). In 1986, another human immunodeficiency virus was identified and designated HIV-2 (Clavel et al., 1986; Montagnier, 2010). Among these primate lentiviruses, HIV-1, HIV-2 and its close relative SIVmac are most well-studied through biological, biochemical and medical approaches, and many findings crucial for the biological and medical sciences have been generated (Ho & Bieniasz, 2008).

Basically, HIV/SIV exhibit a virological phenotype common to the retroviruses. Viral proteins are synthesized from viral DNA genome integrated into host chromosomal DNA, and progeny viral particles (virions) are produced from cells in a typical manner. However, HIV/SIV are unique, as primate lentiviruses, in the genome and virion composition among the retroviruses (Fig. 1). They all have additional genes relative to a standard retrovirus. Importantly, these extra genes encode, in addition to structural Gag, Pol and Env proteins common to all retroviruses, viral regulatory (Tat and Rev) and accessory proteins that are essential for the specific and unique characteristics of HIV/SIV. HIV/SIV virions, therefore, contain some viral proteins not found in the other retroviral virions. The common and unique properties are also applicable to their replication cycle. HIV/SIV replicate in their target cells essentially in the same way with the other retroviruses. Retroviral replication cycle consists of early and late phases. The early phase (Fig. 2) begins with the virion entry step into cells, and proceeds to the reverse transcription of viral RNA genome, uncoating, nuclear import of viral DNA genome, and integration of viral DNA genome into host DNA to generate proviral DNA. The late phase (Fig. 3) then starts with the proviral transcription, and proceeds to the viral RNA export to cytoplasm, translation into viral proteins, assembly of the viral RNA/proteins at cell surface, budding/release from cells, and maturation into infectious virions.

Of viral proteins unique to HIV/SIV, Tat and Rev are essential for virus replication as is the case for structural proteins Gag, Pol, and Env, and act as regulators for expression of the other viral proteins (Freed & Martin, 2007). Tat is a potent trans-activator of transcription, and is the primary switch of viral gene expression. Rev is responsible for the viral RNA export process, and required for expression of viral structural proteins and most of the accessory proteins except for Nef. Thus, Rev can be considered to be the second expression switch. In contrast to the two regulatory proteins, accessory proteins are not always necessary for viral replication in cells (Freed & Martin, 2007). Early studies indicated that these proteins are unnecessary or dispensable for virus replication in the established cell lines. However, it was soon noticed that, in the primary natural target cells such as CD4-positive T-lymphocytes and macrophages, or in some specific cell lines, the accessory

proteins are essential or important for virus replication. These findings have led to the identification of innate anti-viral factors APOBEC proteins (Sheehy et al., 2002) and tetherin as described above (Neil et al., 2008; Van Damme et al., 2008), and to the search for an anti-viral macrophage factor(s) (Fujita et al., 2010). Although some aspects of the accessory proteins are becoming more organized and much clearer than before as summarized in Table 1, detailed mechanisms for their activity remain to be elucidated. In particular, much is still unknown about structurally related Vpr and Vpx proteins. Moreover, functional studies in animals on HIV-1 and HIV-2 accessory proteins have not yet been performed.

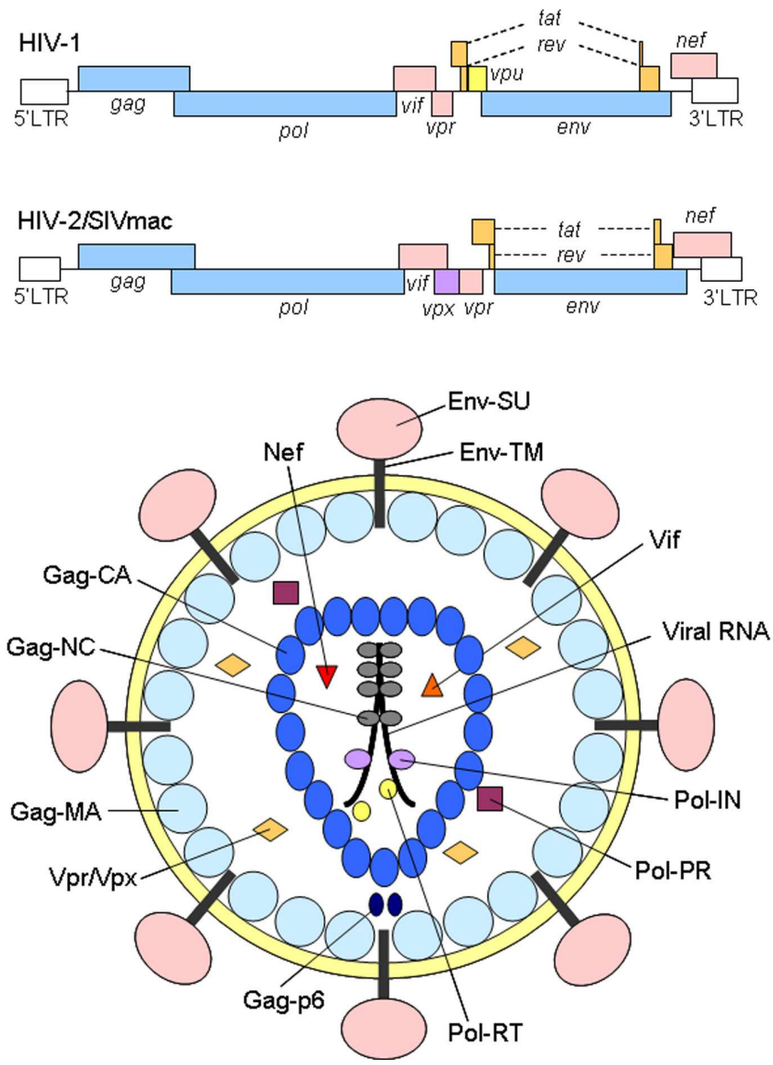

Fig. 1. Genome and virion characteristics of HIV/SIV. Upper: Proviral genome structure is schematically shown. Blue, orange, pink areas (boxes) indicate the structural, regulatory and accessory genes, respectively. Accessory genes unique to HIV-1 (*vpu*) and HIV-2/SIVmac (*vpx*) are indicated by yellow and purple, respectively. LTR, long terminal repeat. Lower: A schema of viral particle (virion). Viral proteins reported to be present in virion are illustrated. CA, capsid; IN, integrase; MA, matrix; NC, nucleocapsid; PR, protease; RT, reverse transcriptase; SU, surface; TM, transmembrane.

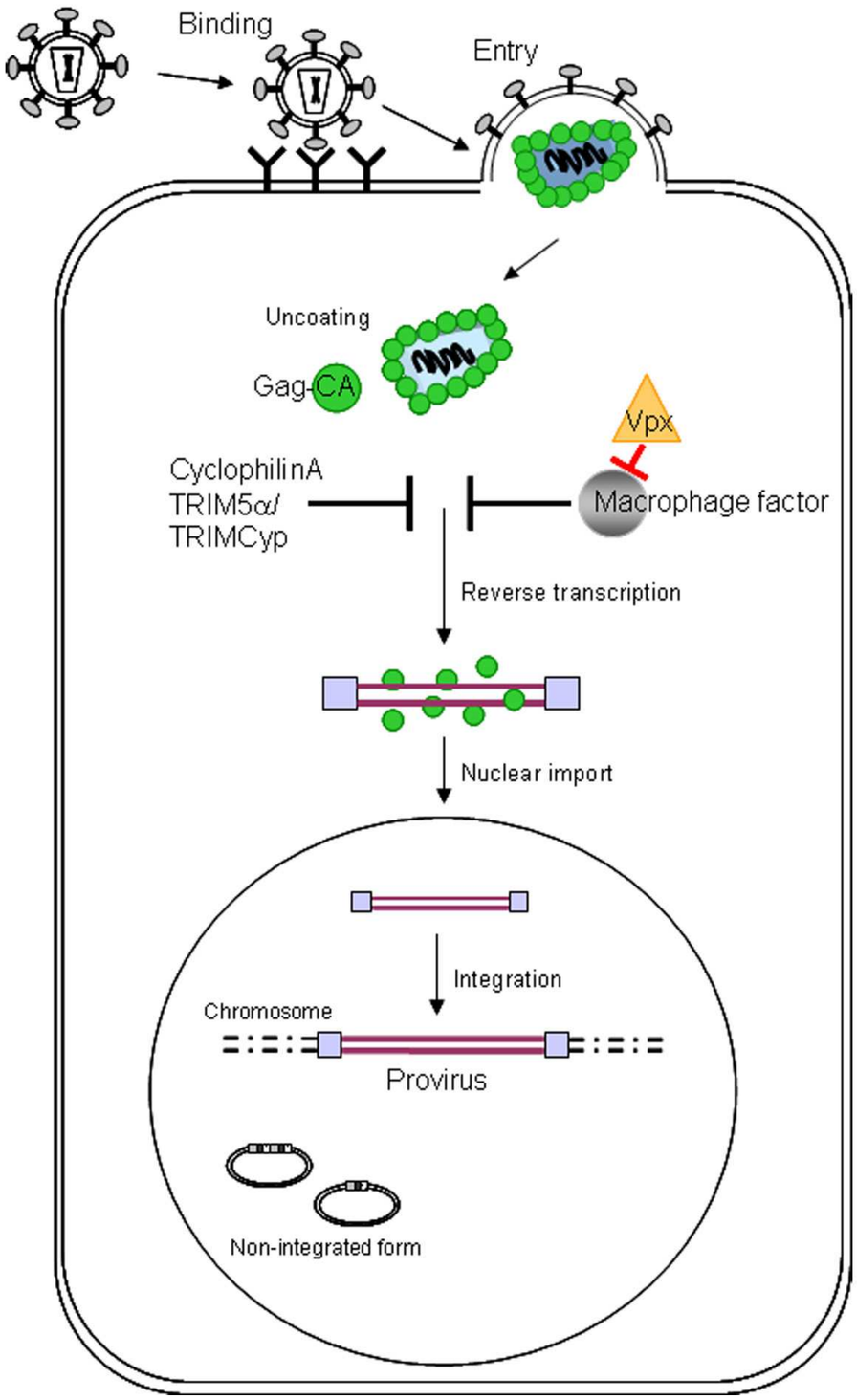

Fig. 2. The early phase of HIV/SIV replication cycle in target cells. Viral replication steps from the binding to generation of provirus are shown. Viral and cellular proteins particularly important in this chapter are highlighted. For details, see the reference (Freed & Martin, 2007).

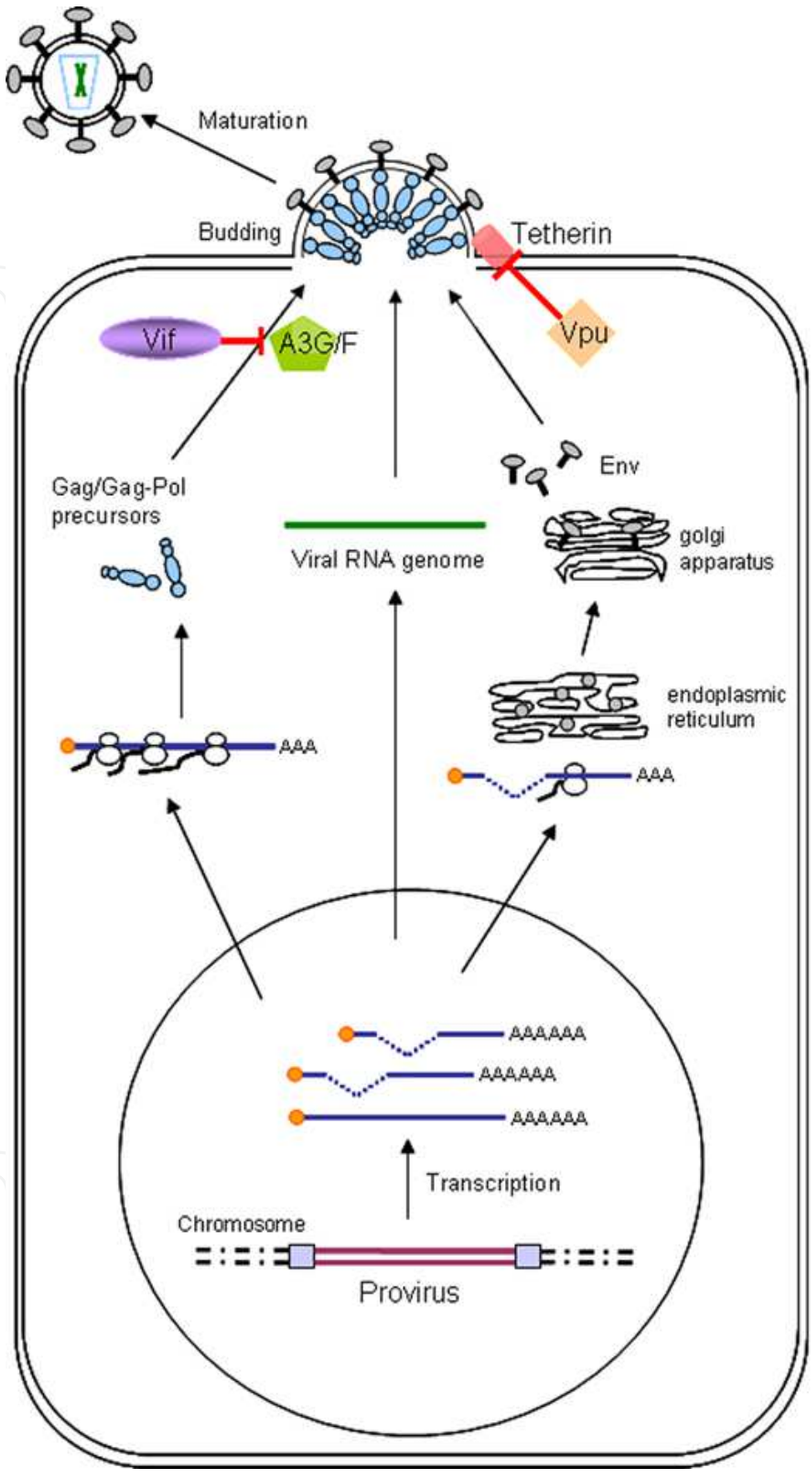

Fig. 3. The late phase of HIV/SIV replication cycle in target cells. Viral replication steps from the transcription of proviral genome to maturation are shown. Viral and cellular proteins particularly important in this chapter are highlighted. For details, see the reference (Freed & Martin, 2007).

Accessory proteins	Activities	References
Vif	Neutralization of antiviral activities of APOBEC3G/F Induction of G2 cell cycle arrest	Holmes et al., 2007 Huthoff & Towers, 2008 Izumi et al., 2010
Vpx	Inactivation of macrophage factor	Fujita et al., 2010
Vpr	Induction of G2 cell cycle arrest Trans-activation of transcription Promotion of nuclear import of pre-integration complex	Le rouzic & Benichou, 2005 Andersen et al., 2008 Ayinde et al., 2010 Fujita et al., 2010
Vpu	Degradation of tetherin Degradation of newly synthesized CD4 in ER	Bour & Strebel, 2003 Nomaguchi et al., 2008b Tokarev et al., 2009
Nef	Down-regulation of cell surface molecules (CD4, MHC-I etc.) Enhancement of viral infectivity	Kirchhoff et al., 2008 Kirchhoff, 2009 Jere et al., 2009

Table 1. Multi-functional activity of HIV/SIVmac accessory proteins. Major functions or activities are listed. For details, refer to the articles shown. ER, endoplasmic reticulum; MHC, major histocompatibility complex.

One of the most outstanding biological properties of HIV-1 is its especially narrow host range. It was recognized soon after the virus isolation that HIV-1 can not infect macaque cells and macaques, animals frequently used for experimental infection. We, therefore, pioneered the work to determine viral determinants for this species-tropism by construction and characterization of chimeric viruses between SIVmac and HIV-1 (Nomaguchi et al., 2008; Sakuragi et al., 1992; Shibata et al., 1991, 1995; Shibata & Adachi, 1992). SIVmac has a wider host range relative to HIV-1, and can efficiently replicate both in macaque and human cells. The chimeric viruses (Fig. 4), later called SHIV, were useful to localize the viral genetic area responsible for the tropism. Among NM-1, NM-3, and NM-8 in Fig.4, only NM-3 was shown to display infectivity to macaque cells. In addition, Gag-CA region was suggested to be important for the tropism by a similar analysis of chimeric viruses (Dorfman & Gottlinger, 1996). Totally, these SHIV studies revealed that Gag-CA plus some viral protein(s) encoded by the central viral genomic region may determine the HIV-1 species-tropism.

As for input viruses of model infection studies in macaques, SIVmac and SHIVs have been widely and frequently used (Ambrose et al., 2007; Nomaguchi et al., 2008). SIVmac is thought to emerge by a cross-species infection of rhesus macaques with SIVsmm naturally occurring in African sooty mangabeys (Fultz et al., 1986; Murphey-Corb et al., 1986). It targets CD4-positive cells such as T-lymphocytes and macrophages, persists, and finally cause AIDS in rhesus macaques. Pathogenic SHIVs have been obtained from the original prototype SHIV by serial animal passages, and were used for infection experiments in rhesus macaques. An SIVmac derivative that has reverse transcriptase (RT) of HIV-1 (RT-SHIV) (Fig. 4) was also constructed to test the effect of anti-RT drugs on virus replication (Uberla et al., 1995). Although these viruses did contribute much to HIV-1 model studies

including the assessment of immune response, evaluation of anti-viral drugs, analysis of drug-resistance, and establishing the strategy for vaccine development, there are some intrinsic differences among important virological properties of HIV-1, SIVmac and SHIVs as summalized in Table 2. These should be seriously considered for the future model studies. To underscore the essential need for the suitable primate model research to answer basic questions about HIV-1 in vivo, we wish to mention here, as an example, that the trials to develop anti-viral vaccines have been unsuccessful due to the lack of appropriate models (Hayden, 2008; Watkins et al., 2008).

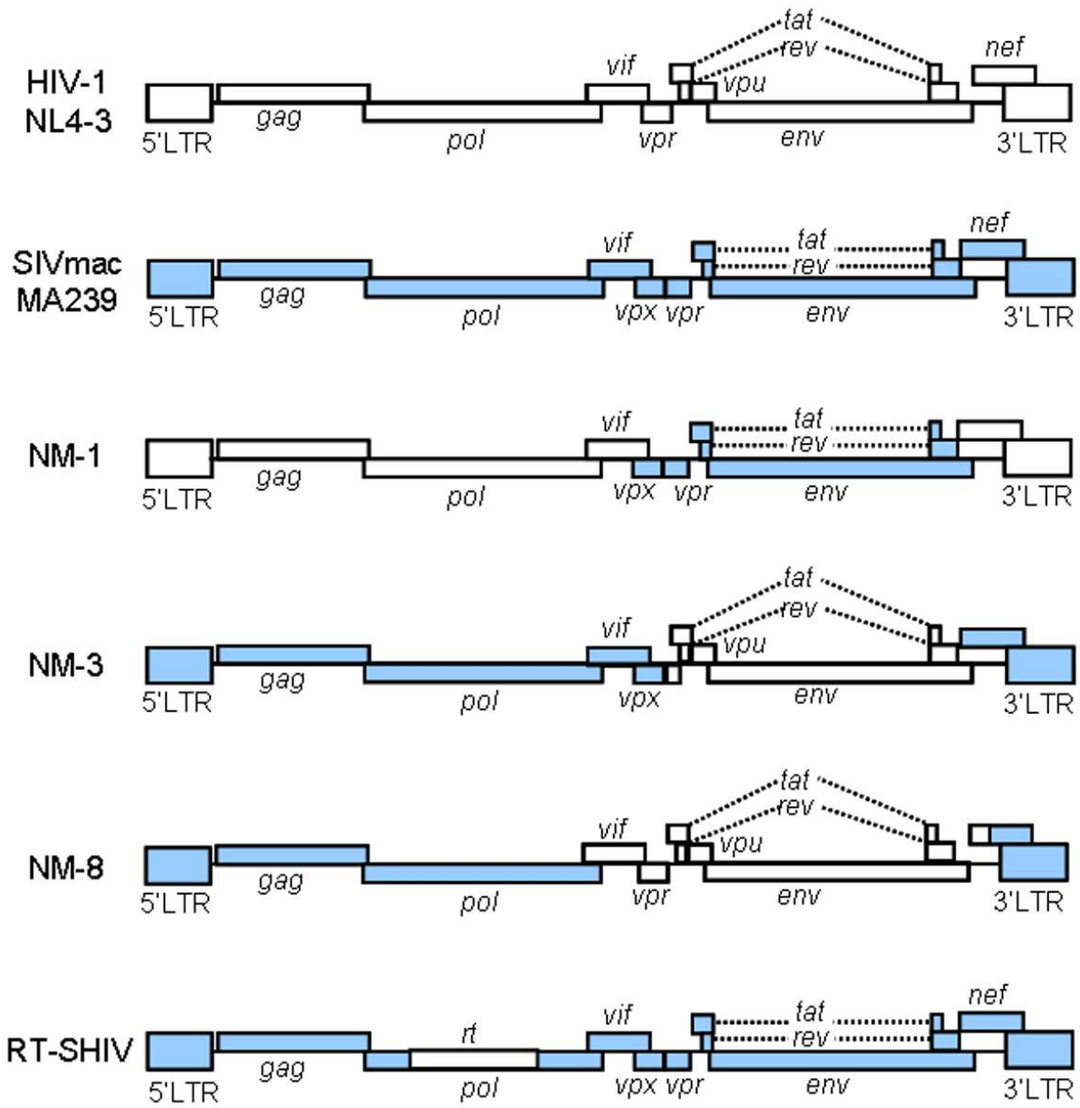

Fig. 4. Genome organization of HIV/SIV and representative SHIVs. Proviral genome structure is schematically shown. White and blue areas (boxes) indicate the genes and LTR of HIV-1 NL4-3 (Adachi et al., 1986) and SIVmac MA239 (Shibata et al., 1991), respectively. Areas without gene names indicate that the genes there are inactivated by genetic manipulations.

	HIV-1/human	SIVmac/rhesus	SHIV/rhesus
Outcomes in individuals			
Response to vaccines			Easily vaccinated
Response to drugs		Not always inhibited by anti-HIV-1 drugs	Not always inhibited anti-HIV-1 drugs
Median disease course	Approximately 10 years	One to three years	Rapid
Emergence of X4-tropic isolates	Frequent (Subtype B)	Rare	
Accessory proteins in cells			
Vpx	Not present	Inactivate an unidentified anti-viral factor in macrophages	
Vpr	Influence viral replication in macrophages	Act on viral replication nothing in macrophages	
Vpu	Antagonize tetherin	Not present	
Nef	Not antagonize tetherin Not down-regulate CD3	Antagonize tetherin Down-regulate CD3	

Table 2. Biology of HIV-1, SIVmac, and SHIV in infected hosts. For details, see the main references

3. Determinants for HIV-1 species-tropism

Our early studies on systematic analysis of HIV-1 proviral mutants by site-directed mutagenesis have clearly demonstrated the cell-dependent functionality of some viral proteins (Gag-CA, Vif, Vpu, and Vpx) and the cell-dependent viral replication (Adachi et al., 1999; Kawamura et al., 1994b, 1998; Sakai et al., 1993, 1995; Sakuragi et al., 1995). These results have strongly suggested the presence of specific intracellular factors, other than receptor molecules for viruses, responsible for viral cellular tropism. Importantly, restriction factors against HIV-1 (APOBEC3/Vif, TRIM5α/Gag-CA, and tetherin/Vpu) have been recently identified and molecularly cloned (Neil et al., 2008; Sheehy et al., 2002; Stremlau et al., 2004; Van Damme et al., 2008). Furthermore, a new restriction factor functional in macrophages and antagonized by Vpx has been proposed (Fujita et al., 2008; Fujita et al., 2010; Sharova et al., 2008; Srivastava et al., 2008). Taken altogether, these findings have prompted active researchers to examine whether these cellular proteins are associated with the HIV-1 species-tropism. As results of a series of comparative biological and biochemical studies on the interaction between HIV/SIV and human/monkey restriction factors, it has been revealed that various species-specific cellular proteins in Table 3 determine or modulate the species-tropism of HIV-1. As can be understood in Table 3, viral accessory proteins Vif, Vpu, Vpx (and/or Vpr), and Nef (in the case of some SIVs) play significant roles (Tables 1 and 2) against the restriction factors present in host cells (Malim & Emerman,

Host restriction factors	Viral proteins	Antiviral effects
APOBEC3G/F	Vif	Induction of lethal mutations in the viral genome
CypA and TRIM5α/TRIMCyp	Gag-CA	Block of post-entry replication steps
Tetherin/BST-2	Vpu	Inhibition of virion release
Macrophage factor?	Vpx/Vpr?	Suppression of uncoating / reverse transcription?

Table 3. Restriction factors against HIV-1. Cellular anti-HIV-1 factors identified and one of potential anti-viral factors are listed. As for the details of restriction factors of these two categories, see the text.

2008). It is well-predicted that primate immunodeficiency viruses now have evolved by acquiring the appropriate accessory genes through numerous mutations and recombinations (Kirchhoff, 2009, 2010; Sauter et al., 2009, 2010). Among viral structural proteins, only Gag-CA, which constitutes a major virion component, appears to be deeply involved in the species-tropism of HIV-1. By adapting Gag-CA and accessory proteins to the hostile environment, HIV/SIV could spread, persist, and survive. In this regard, HIV-1 has developed its specific characteristics from the progenitor form, and may be still uniquely altering its virological property through multiple rounds of the infection cycle in human populations.

3.1 Vif and APOBEC3G/F

Accessory protein Vif (Table 1) is essential for HIV/SIV replication in certain cell types such as natural target cells (T-lymphocytes and macrophages) that express APOBEC3G/APOBEC3F. APOBEC3G/F are members of a polynucleotide cytidine deaminase family that displays

diverse functions (Holmes et al., 2007), and are potent inhibitors of viral replication counteracted by Vif. Vif degrades APOBEC3G/F via the ubiquitin-proteasome pathway (Table 1 and Fig. 3). In the absence of Vif, APOBEC3G/F are incorporated into virions, and cause lethal mutations in viral genome during the reverse transcription process in a new infection cycle (Table 3). There are two functional domains in Vif, that is, N-terminal binding region to APOBEC proteins and C-terminal region for degradation (Strebel et al., 2009). Noteworthy, HIV-1 Vif does not degrade APOBEC3Gs of the rhesus macaque and African green monkey probably due to its inability to binding to them. In contrast, SIVmac Vif can inactivate both human and simian APOBEC3Gs. Thus, the interaction of Vif and APOBEC3G/F is critically important for the unique species-tropism of HIV-1. In our experience, APOBEC3G/F is the strongest determinant for this tropism among the restriction factors listed in Table 3. Whether another activity of Vif to induce G2 cell cycle arrest (Izumi, T., 2010) (Table 1) is involved in the species-tropism is presently unknown.

3.2 Gag-CA and its interacting cellular proteins (CypA, TRIM5α and TRIMCyp)

Early studies have already indicated that Gag-CA is responsible for the HIV-1 species-tropism as described above (Shibata et al., 1991; Dorfman & Gottlinger, 1996). Recent works have focused on the interaction of Gag-CA and its counterpart (CypA, TRIM5α and a TRIM5α/CypA fusion protein, TRIMCyp). It is well-established now that CypA, TRIM5α and TRIMCyp act as an inhibitor of HIV-1 replication in a species-specific manner (Lim et al., 2010; Luban, 2007; Nakayama & Shioda, 2010; Price et al., 2009; Towers, 2007; Ylinen, 2010). These cellular proteins exert their anti-viral powers on the incoming virion core in a poorly defined way (Table 3 and Fig. 2). Of note, CypA positively and negatively regulates HIV-1 replication in human and macaque cells, respectively. Importantly, rhesus TRIM5α, cynomolgus TRIM5α and cynomolgus TRIMCyp effectively inhibit HIV-1 replication, but not rhesus TRIMCyp. Therefore, CypA, TRIM5α and TRIMCyp can determine the unique species-tropism of HIV-1. We estimate that Gag-CA is the second strongest determinant for the tropism. It should be stressed here that the polymorphism observed in TRIM5 alleles affects the sensitivity of hosts to virus infection.

3.3 Vpu and tetherin

Accessory protein Vpu (Table 1) is required for optimal replication of HIV-1 in certain cell types that express tetherin. Tetherin specifically inhibits the virion release from cells (Table 3) and is countered by Vpu (Nomaguchi et al. 2008b; Strebel et al., 2009). Vpu degrades cellular tetherin and CD4 effectively. It is generally accepted that Vpu enhances virion release from the cell surface by down-regulation of tetherin (Table1, Table 2 and Fig.3), and thereby promote viral replication. However, Vpu proteins of HIV-1 and some SIVs can not efficiently antagonize simian tetherin molecules relative to those of SIVs with a high ability (Sauter et al, 2009). In fact, HIV-1 NL4-3 scarcely suppressed the anti-viral activity of the rhesus tetherin. Based on this finding, it can be concluded that tetherin is associated with the species-tropism of HIV-1. However, in our experience, the positive effect of Vpu on viral replication is much smaller than those of Vif and Gag-CA. Moreover, another functional activity of Vpu to degrade cellular CD4 is considered to be irrelevant to the HIV-1 species-tropism. Whether Vpu is associated with the HIV-1 pathogenesis is an important question to address. Interestingly and importantly, Env of some HIV-2 isolates and Nef of some SIVs have the Vpu-like ability to enhance virion release (Strebel et al., 2009; Zhang et al., 2009).

3.4 Potential determinants for HIV-1 species-tropism

It has been recently reported that HIV-2/SIVmac Vpx is necessary for the post-entry step of viral replication, such as uncoating/reverse transcription, in monocyte-derived dendritic cells and macrophages (Fujita et al., 2008; Goujon et al., 2007; Srivastava et al., 2008). Vpx is supposed to counter an unidentified anti-retroviral factor(s) present in cells of this lineage (Tables 1-3 and Fig. 2). Because Vpx can also up-regulate the HIV-1 replication, the unidentified macrophage factor appears to be commonly important for HIV/SIV replication. To substantiate the macrophage entity as a restriction factor against HIV/SIV and/or the other retroviruses, its identification is urgently required.

During a systemic characterization of HIV-1mt CA mutants, we have noticed a TRIM5α-independent enhancement of viral infectivity in macaque cells (Nomaguchi et al., manuscript in preparation). This result suggests the presence of unknown anti-viral factor that interact with HIV-1 Gag-CA. We also have found a mutation in the Env-SU region that confers the mutant a significant affinity to macaque CD4, considerably promoting virus replication (Nomaguchi et al., manuscript in preparation). These observations may be relevant to the HIV-1 species-tropism.

4. Generation and characterization of various HIV-1mt clones

To obtain a novel class of HIV-1 that infects, replicates and finally causes AIDS in macaques, we and a research group in USA have independently initiated the work on HIV-1mt and have done macaque model studies (Hatcho et al., 2008; Hatziioannou, 2006, 2009; Igarashi et al., 2007; Kamada et al., 2006, 2009; Kuroishi et al., 2009; Nomaguchi et al., 2008a; Saito et al., 2011; Yamashita et al., 2008). Another group has published a report on HIV-1mt derivatives very recently (Thippeshappa et al., 2011). We now are actively and thoroughly amending the HIV-1mt genome by computer-assisted and structure-guided mutagenesis.

Our prototype HIV-1mt designated NL-DT5R (Kamada et al., 2006) contains a 21-nucleotide SIVmac Gag-CA element (corresponding to the HIV-1 CypA-binding loop) and the entire SIVmac *vif* gene inserted into the genetic background of HIV-1 NL4-3 (Adachi et al., 1986). From this clone, we have systemically generated a series of HIV-1mt clones as shown in Fig. 5. Because CCR5-tropic (R5) viruses of HIV-1 are thought to be clinically more important than CXCR4-tropic (X4) viruses, we have constructed two sets of HIV-1mt clones. Our strategy for generation of HIV-1mt clones pathogenic for macaques are as follows: (i) Adaptation of viruses in macaque cells. Targets for infection are cynomolgus and rhesus macaque lymphocyte cell lines immortalized by Herpesvirus saimiri (HVS) (Table 4).; (ii) In vitro mutagenesis of the clones based on bioinformatics. With the aid of the computational sciences, new viral genome sequences are designed.; (iii) Selection of appropriate clones by their replication kinetics in macaque lymphocyte cell lines in Table 4. Viruses which replicates similarly with or more robustly than SIVmac239 in cynomolgus and rhesus peripheral blood mononuclear cells are then chosen. On the basis of this strategy, we have successfully obtained a number of new generations with increasing ability to replicate from the original prototype NL-DT5R (see below). However, so far, none of the HIV-1mt clones tested are pathogenic for macaques (pig-tailed and cynomolgus) (Igarashi et al., 2007; Nomaguchi et al., manuscript in preparation; Saito et al., 2011), although they all can replicate in the monkeys. The newest clones in Fig. 5 (MN4Rh-3V and MN5Rh-3V), which replicate best in macaque cells among our HIV-1mt clones, have not yet been examined for

their pathogenicity. It should be mentioned here that the replication potentials of the HIV-1mt clones in cell lines parallel with those in individuals.

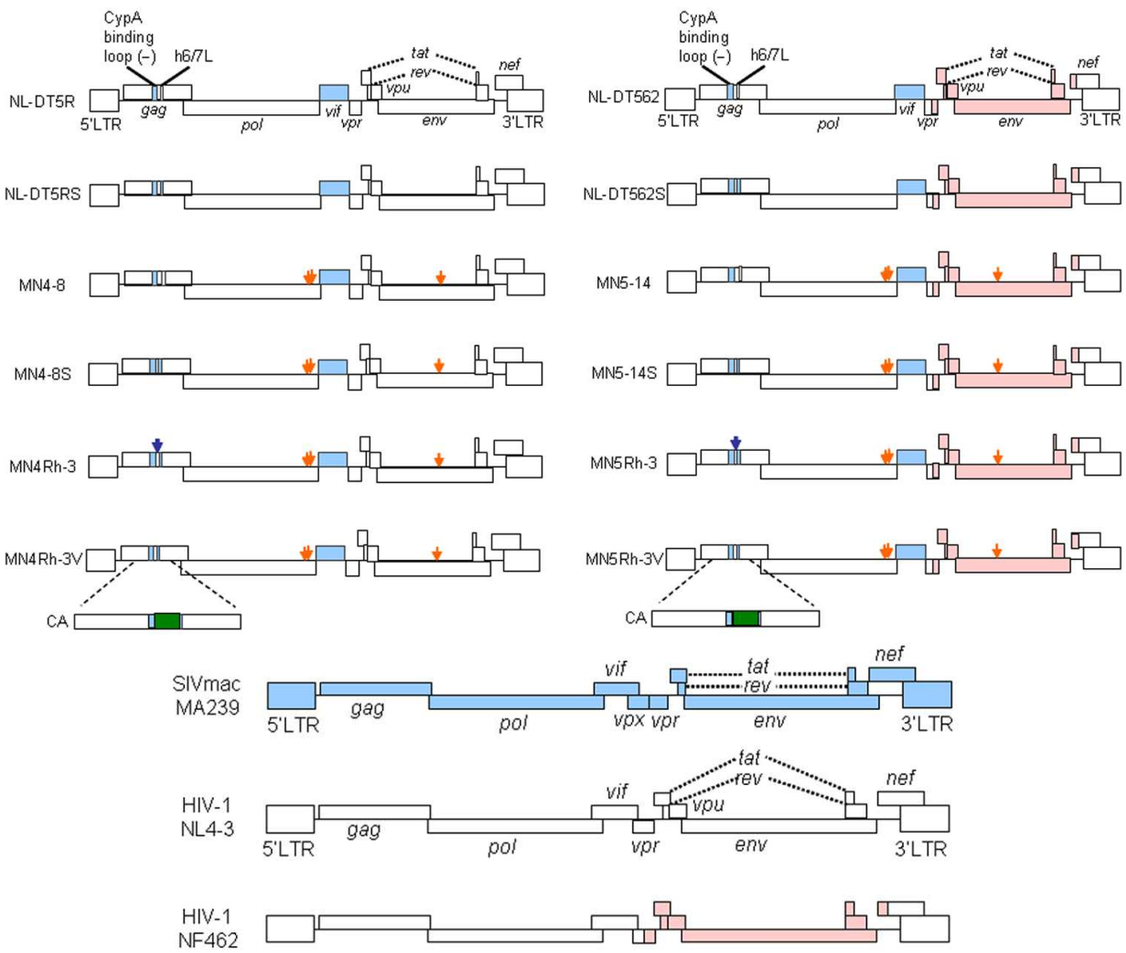

Fig. 5. Genome organization of HIV/SIV and various clones of HIV-1mt. Proviral genome structure is schematically shown. Blue, white and pink areas (boxes) indicate the genes and LTR of SIVmac MA239 (Shibata et al., 1991), X4-tropic HIV-1 NL4-3 (Adachi et al., 1986) and R5-tropic HIV-1 NF462 (Kawamura et al., 1994a), respectively. HIV-1mt clones on the left and right are X4 and R5 viruses, respectively. Arrows indicate the site of each single/double nucleotide-mutation introduced (Nomaguchi et al., manuscripts in preparation). There are several single-nucleotide mutations in the green area of Gag-CA (Nomaguchi et al., unpublished). h6/7L, Loop between helices 6 and 7.

In parallel with the generation and characterization of a series of HIV-1 mt clones, we have searched for and established macaque cell lines suitable for our projects. Table 4 lists the cell lines we routinely use now. Since the lymphocyte cell lines immortalized by HVS do not lose their original characteristics as primary lymphocytes in most cases and are readily maintained for experiments, to biologically characterize viruses like HIV-1, it is quite important for laboratory researchers to have HVS-immortalized cell lines. In our laboratory, cynomolgus HSC-F (Akari et al., 1996; Fujita et al., 2003) and rhesus M1.3S (Doi et al., 2011)

cell lines are chosen as targets for virus infection, and frequently used. HSC-F cells are very sensitive to HIV-1mt and SIVmac clones, and produce a large amount of progeny viruses after infection. M1.3S cells are quite resistant to HIV-1mt and SIVmac clones, and are appropriate for selection of highly replicable and potentially pathogenic viruses. Because we are interested in analyzing the species-tropism of HIV-1, we need to have various target cell lines of human and simian origins with a unique property. Monolayer cell lines of cynomolgus MK.P3 (F) and rhesus LLC-MK2 are easily used for transfection experiments and for monitoring the single-cycle viral infectivity assays. In fact, we have differentially and successfully used the cell lines in Table 4 depending on the purpose of each project.

Macaques	Cell lines	Origins	TRIM5 alleles
Cynomolgus	HSC-F	lymphocyte	TRIM5α and TRIMCyp
	MK.P3 (F)	kidney	TRIM5α and TRIMCyp
Rhesus	HSR1.4	lymphocyte	Mamu-3 and Mamu-4
	HSR5.4	lymphocyte	Mamu-7
	M1.3S	lymphocyte	Mamu-1 and Mamu-3
	LLC-MK2	kidney	Mamu-1 and Mamu-7

Table 4. Cell lines for virological evaluation of HIV-1mt. TRIM5 alleles of the cell lines listed have been determined in our laboratory (Doi et al., 2010; our unpublished results). For the polymorphism of TRIM5 alleles, see the references (Newman et al., 2006; Virgen et al., 2008; Wilson et al., 2008).

We have repeatedly examined the replication kinetics of HIV-1mt clones in various macaque cell lines. Fig. 6 shows the typical kinetics (a schema) based on the results from our numerous infection experiments. In highly sensitive HSC-F cells, all the viruses do replicate to distinct extents. As is clear, MN4Rh-3V and MN5Rh-3V replicate most robustly among HIV-1mt clones. In relatively resistant M1.3S cells, three clones do replicate but the others do not. In both cell lines, SIVmac239 (MA239N) (Doi et al., 2010) displays the best potential to replicate. These results indicate that we still need to improve MN4Rh-3V and MN5Rh-3V to obtain the ideal clone, the pathogenic HIV-1mt. In this situation, there are two directions. These are the selection of host macaques susceptible to the currently available clones and the further efforts to obtain the desired clones. First, pig-tailed and/or the other macaque species sensitive to the viruses can be selected by their TRIM5 alleles (Newman et al., 2006; Virgen et al., 2008; Wilson et al., 2008), and used for infection. Indeed, American research groups have adopted this strategy using the pig-tailed macaques/variants of simian-tropic (st) HIV-1 with a *vif*-substitution only (Hatziioannou et al., 2009; Thippeshappa et al., 2011). However, we very much prefer to take the second possibility. Through this approach, we would be able to better understand the molecular mechanism underlying various events between the pathogen and host. Furthermore, if one is interested in the studies to analyze the mutations, adaptations, and evolution of the pathogen, the pressure-giving environment (Malim & Emerman, 2008), i.e., natural hosts having a wide variety of restriction factors, would be much better. Of a particular note, pig-tailed monkeys infected with various st HIV-1s have not yet develop AIDS (Igarashi et al., 2007; Hatziioannou et al., 2009; Thippeshappa et al., 2011).

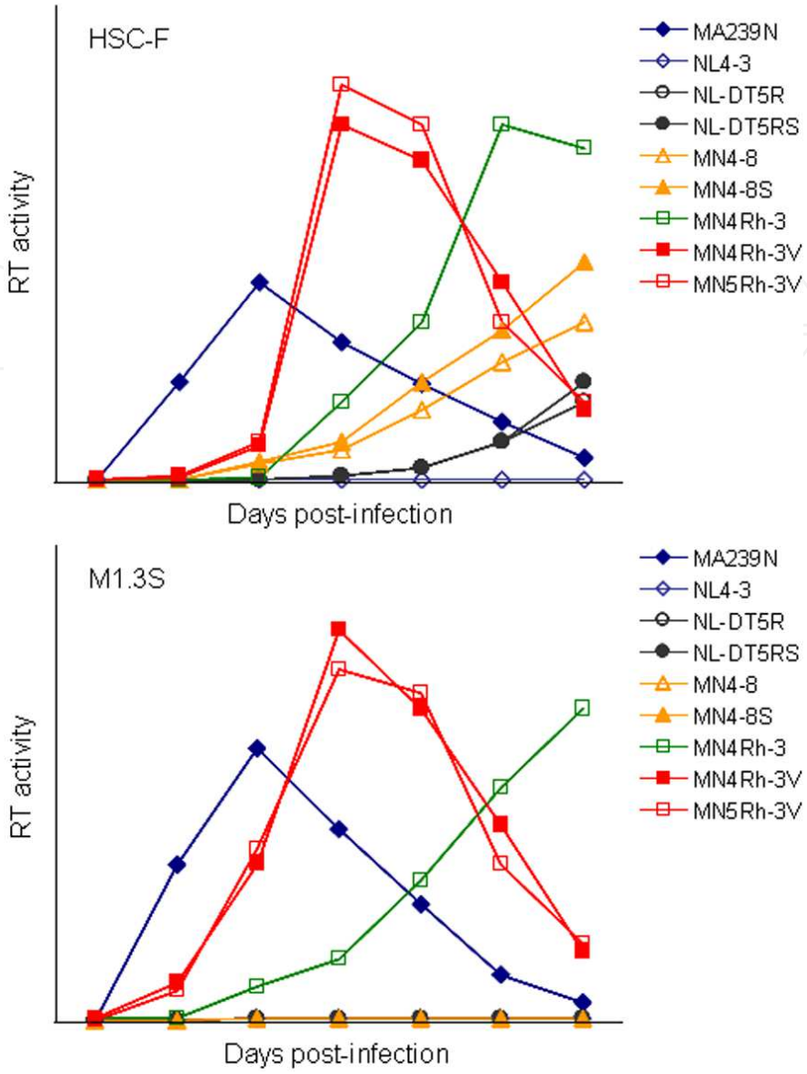

Fig. 6. Schematic representation of replication kinetics of various viral clones. A schema of replication kinetics is illustrated. Molecular proviral clones for study are shown on the right. Routinely, cell-free virus samples are prepared by transfection of proviral clones into 293T cells (Kamada et al., 2006), and viruses produced in cells of equal RT units are inoculated into HSC-F and M1.3S cells (Table 4). After infection, viral replication is monitored at intervals by RT activity in the culture supernatants.

5. Conclusion

We have described the generation of CXCR4-tropic and CCR5-tropic HIV-1 clones with macaque cell-tropism (HIV-1mt) in this chapter. The best X4 and R5 viruses we have now replicate comparably with a standard SIVmac clone in macaque cells, although their pathogenicity for macaques needs to be determined. The genomes of these HIV-1 mt clones contain the entire *vif* gene of SIVmac, some nucleotide substitutions in the *gag* gene to give a small number of mutated amino acids, two adaptive mutations in the *pol* gene, and one adaptive mutation in the *env* gene (Fig. 5).

For the moment, our goal is to have the HIV-1mt clones pathogenic for cynomolgus and/or rhesus macaques with the aid of computational sciences. The clones are expected to have the HIV-1-derived or closely related accessory genes except for the *vif* gene. With these ideal HIV-1mt clones, we would be able to authentically investigate the HIV-1/host interaction including: (i) viral replication in individuals; (ii) viral pathogenesis; (iii) viral mutations/adaptations/evolution. Once these clones are available, a wide variety of basic and clinical studies would be initiated otherwise impossible.

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Many original articles reporting the scientifically new and important findings could not be cited due to the tremendous numbers of publications and the space limitations. We express our sincere regret over these omissions based on rather subjective considerations.

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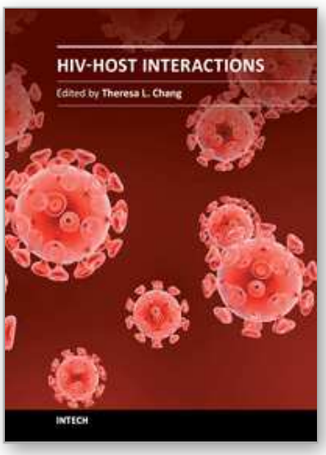

HIV-Host Interactions

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HIV remains the major global health threat, and neither vaccine nor cure is available. Increasing our knowledge on HIV infection will help overcome the challenge of HIV/AIDS. This book covers several aspects of HIV-host interactions in vitro and in vivo. The first section covers the interaction between cellular components and HIV proteins, Integrase, Tat, and Nef. It also discusses the clinical relevance of HIV superinfection. The next two chapters focus on the role of innate immunity including dendritic cells and defensins in HIV infection followed by the section on the impact of host factors on HIV pathogenesis. The section of co-infection includes the impact of Human herpesvirus 6 and Trichomonas vaginalis on HIV infection. The final section focuses on generation of HIV molecular clones that can be used in macaques and the potential use of cotton rats for HIV studies.

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