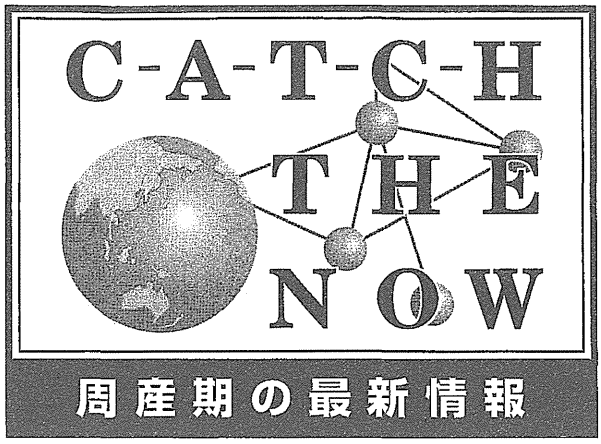

2011年から全妊婦に対して妊婦健診でHTLV-1抗体検査が行われるようになった．今後，キャリア減少を目指して取っていくべき方策と，キャリア妊婦へのかかわり方など，母子感染対策のために産科スタッフが知っておきたい知識を解説する．

HTLV-I母子感染対策のために助産師が知っておきたい知識

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はじめに

これまで九州・沖縄など，ごく一部の地域で行われてきたHTLV-I母子感染対策が，2011年から全国で行われるようになった．2011年度からは全国の都道府県でHTLV-I母子感染対策協議会が設置される予定で，各地域での保健指導およびカウンセリング体制の検討，市町村職員などへの研修，HTLV-I母子感染対策に関する普及啓発が行われることになっている．この中で保健所，女性健康支援センターでは授乳に関する指導，助言や不安や悩みのカウンセリングを担当し，産婦人科医療機関では，妊婦のHTLV-I抗体スクリーニング，確認検査の実施，結果の説明の後に授乳に関する指導，助言を行うことになっている[1]．HTLV-Iは後述するように成人T細胞白血病（ATL）やHTLV-I関連脊髄症（HAM）などの難治性の疾患を，キャリアのごく一部に引き起こすが，母乳を介して母子感染することが知られている[2, 3]．母子感染を防ぐために母乳を制限することを指導するが，従来のように断乳の上，人工乳で育てる方法以外に，母乳を搾乳していったん凍らせてから，解凍して哺乳瓶で与える凍結母乳法[4]，3カ月までの短期母乳も母子感染を減少させることが分かっている[5]．臨床現場でHTLV-Iキャリア妊婦に母乳哺育法を直接指導する立場にある助産師や看護師にも，HTLV-Iの知識は必須事項となっており，また不安や悩みのカウンセリングにも対応することが今後増してくると思われる．本稿ではHTLV-I母子感染について概説する．

HTLV-Iについて

1981年に京都大学の日沼頼夫らにより，成人T細胞白血病（ATL）の原因ウイルスとしてHTLV-Iが発見された[6]．このウイルスはCD4陽性のT細胞に感染し，いったん感染すると，生涯にわたりウイルスを持ち続ける（キャリアという）．HTLV-Iキャリアは1990年ごろは120万人と推定され，九州・沖縄にキャリアの大半が居住していた[7]．その後，2006年，2007年の全国調査（厚生労働研究：山口班）で，キャリア数は108万人と少し減少したが，キャリアの居住地が九州・沖縄から，全国に拡散していることが明らかとなった（表1）[8]．重要なことと

表1　HTLV-Iキャリア推定数（献血者からの陽性率から推定）

地　域	1990年		2006年，2007年	
	キャリア数	キャリア地域別（%）	キャリア数	キャリア地域別（%）
北海道・東北	108,000	9.1	74,753	6.9
関東（東京）	128,300	10.8	190,609	17.7
北陸・東海	82,100	6.9	81,802	7.6
近　畿	202,300	17.0	171,843	15.9
中国・四国	65,000	5.4	67,133	6.2
九州・沖縄	607,300	50.9	492,582	45.7
全　国	1,193,000	100.0	1,078,722	100.0

平成2年度厚生省成人T細胞白血病（ATL）の母子感染防止に関する研究（重松班），平成20年度厚労省研究本邦におけるHTLV-I感染及び関連疾患の実態調査と総合対策（山口班）のデータを一部改変

して，HTLV-Iキャリアのすべてが ATL や HAM を発症するのではなく，ATL だと40歳を過ぎたころから毎年キャリア1,000人に1人くらいの発病（生涯発病率は約5%），HAM では生涯発病率は0.25%と低率であることを認識する必要がある．HTLV-Iウイルスの感染を家系調査したところ，家族内集積が認められ，母親がキャリアであれば高率に子どもに感染することが知られていた[9]．その後の詳細な疫学調査，基礎研究により，HTLV-Iは母乳を介して子どもに感染することが判明した．そのほかの感染ルートとして，輸血感染，性行為を介した夫婦間感染があるが，輸血感染に関しては，1986年より献血時に感染症スクリーニングを行っているため，現在は皆無である．ATL の発病には，感染してから数十年を要することから（平均発症年数は58歳である），ATL 患者のすべては母子感染例である．従って ATL を撲滅するためには母子感染対策が極めて重要になる．

国はこれらのことを受け，HTLV-Iを地方の風土病ではなく，全国に広がるウイルスとして認め，母子感染を防ぐために，妊婦に対する HTLV-I 抗体検査を2010年度より公費負担とし，各都道府県に HTLV-I 母子感染対策協議会を設置し，スムーズに母子感染対策が行われるように，各都道府県に働き掛けた．

母乳を介した母子感染

HTLV-I は CD4 陽性 T リンパ球（免疫を司るリンパ球）の中に潜んでおり，血漿中には存在しない．感染したリンパ球が，非感染リンパ球と接触することにより感染が生じる（図1）．感染は生きたリンパ球同士でないと起こらないので，いったん母乳を凍らせると母乳中のリンパ球は死んでしまうため，感染が起こらなくなる．また母体血中には感染を中和する抗体が存在する．これらの抗体（IgG，IgM，IgA，IgE）の中の IgG 抗体は，胎盤を通過して胎児に移行するため，胎児は生まれた際，母体と同量の感染中和抗体を持っている．母体由来の感染中和抗体の半減期は1カ月であるため，出生後3カ月ま

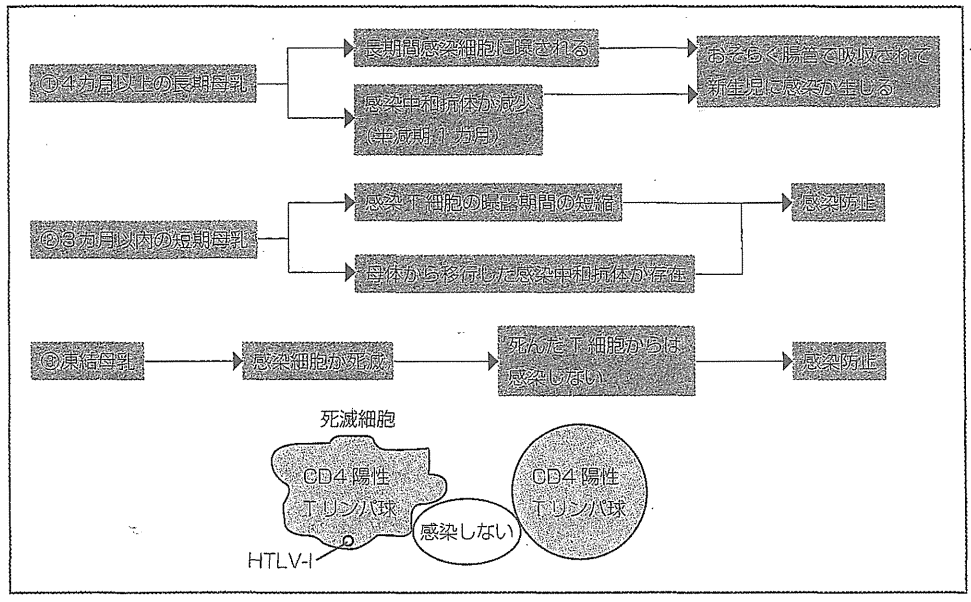

図1 HTLV-I 感染様式

では感染中和抗体は，少なくとも8分の1以上存在する．3カ月までの短期授乳だと，感染曝露期間も短くなり，中和抗体も存在するため母子感染が生じにくい（図1）．

表2に1990年までのHTLV-I母子感染率のデータと1990年以降のデータを示す[5]．1990年ごろまでは，HTLV-I母子感染防止のためには，人工哺育の選択肢しかなかったが，人工乳では母乳哺育に比べて母子感染率が明らかに減少している．1990年以降は，凍結母乳，3カ月までの短期母乳でも十分に人工乳と同程度の母子感染防止効果があることが判明した[5]．このため2011年発刊の『産婦人科診療ガイドライン　産科編2011』では人工哺育，凍結母乳，3カ月までの短期母乳のいずれかを勧めている[10]．しかし，一方的に勧めるのではなく，妊婦の意思を尊重する．母子感染のリスクを承知で強く母乳哺育を希望する際は，妊婦の希望に添うように対応する．

完全人工栄養，3カ月までの短期母乳，凍結解凍母乳法のメリットとデメリット

HTLV-Iキャリア妊婦ならびに夫に，上記3つの栄養法を提示した際，必ず各栄養法のメリットとデメリットを質問される．

人工栄養は，感染したリンパ球を子どもが飲むことがなく，これまでに1,500例以上のデータがあり，最も確実に母子感染を防ぐことが証明されている方法である．しかし，約3%に母子感染以外の感染ルートが見られたことから，完全には母子感染を予防できない．また，母子間の母乳哺育を介したスキンシップ，愛情形成が不十分となるデメリットがある．そのほか，母乳中に含まれるIgA抗体が補給され

表2　HTLV-I母子感染率

〈1990年まで〉
- 母乳哺育：103/788（13.1%）
- 人工哺育：36/953（3.8%）

〈1990年以降〉
- 母乳哺育
  4カ月以上：93/525（17.7%）
  3カ月以下：3/162（1.9%）
- 人工哺育：51/1,553（3.3%）
- 凍結母乳：2/64（3.1%）

ないデメリットもある．人工乳の際は，しっかりと子どもを抱きしめ，目を見つめるようにして哺乳するように指導してほしい．また，母乳をあげたいのに母乳をやめたお母さんのその気持ちは，子どもに対する強い愛情であることをカウンセリングしていただきたい．筆者の経験では，人工乳哺育を行ったために母子関係がうまく形成されなかったということはない．

凍結解凍母乳哺育では，まず搾乳を行い，母乳パックに母乳を移してから，1晩から1日間，家庭用冷凍庫（－18度）で母乳を凍らせる．凍結することで感染リンパ球は死滅し，感染性が消失するので，しっかりと凍っていることを確認する．その後，ぬるま湯（37度前後）で解凍し，母乳が人肌の温度になるまで温めて哺乳瓶で哺乳させる．栄養学的には母乳と全く同じなので，IgA抗体も補給される上に，3カ月以上の長期母乳も可能である．ただし手間がかかることと，直接母乳を与えることができないという不満感はある．しかし，この方法は理論的にも妥当性があり，母乳の持つ栄養成分を損なわずに栄養できるので，もう少し普及してもよい方法と思われる．

3カ月までの短期母乳は人工乳と同等の感染予防効果を有し，かつ直接自分の乳房から哺乳できるため，母親の満足感も高く，母子間愛情形成にも有利に働く．ただし，症例数が少ないことと，途中で母乳哺育をやめられずズルズルと長期母乳になるケースが散見されることがある．2カ月の終わりごろ，もしくは3カ月に入ったころより徐々に粉ミルクと母乳の混合栄養にしていき，3カ月末には母乳から人工乳に切り替えることが重要である．また3カ月まで母乳を与えた場合，薬剤で母乳分泌を止めることは難しい．そのため，しばらくは乳房緊満は続くが，乳房を冷やしたりして対応する．4カ月目から凍結母乳に切り替えることは可能かと質問されることがあるが，この点にはいまだエビデンスはない．おそらく感染のリスクは増加しないと思うが，実行される場合は，いまだエビデンスにはなっていないと説明した上で，行っていただきたい．

HTLV-Iキャリアと説明した後の対応

図2に示すように，妊娠30週ごろまでにHTLV-I抗体スクリーニング法を公費で行う．陽性となった場合，偽陽性である可能性があるので，必ず確認検査が必要であると説明した上で，ウエスタンブロット（WB）法を保険診療で行う[5]．九州・沖縄などの一部の地域を除いては，一次検査で陽性となった症例の20～30%のみが，真の陽性（キャリア）である．そのため一次検査の結果のみでキャリアと判断せずに，必ずWB法を行い，陽性であれば，その時点でキャリアと説明し，十分な時間をとって対応，説明する[5, 10]．WB法陰性の場合は，母乳哺育を勧める．ただし，WB法で10～20%に

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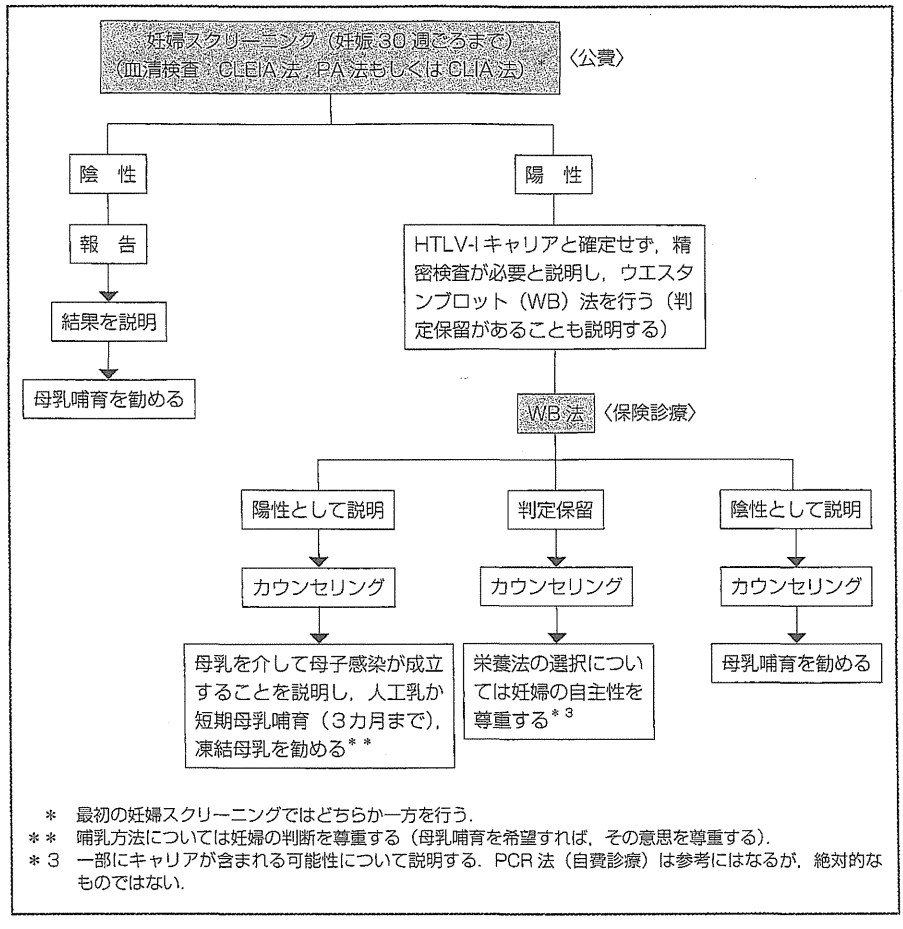

図2　HTLV-Iスクリーニングの進め方

判定保留となるケースがある．このような場合，自費診療となるがPCR法で精査することも可能である[5]．しかし，高価な検査であるため，厚生労働研究：板橋班，浜口班が検査会社と協力して無償で判定保留例にPCR法を行い，その結果を伝える事業を始めたので，これを利用いただきたい．余ったサンプルで，PCR法の評価を7カ所の施設で行い，将来HTLV-I PCR法が保険適用される際の試料とさせていただく．また血漿は判定保留の少ないWB法の確立に役立てる計画である．

典型的なHTLV-Iキャリア

表3に示すように，まず自分がHTLV-Iキャリアであることに大きなショックを受ける．HTLV-Iキャリアのすべてが ATL，HAMになるのではなく，40歳を過ぎてからキャリア1,000人に1人の割合で発症する（喫煙による肺癌の発癌の半分程度）と話し，無用の心配をしないよう説明するが，この際，カウンセリングが必要なこともある．

表3　典型的なキャリア例

1. HTLV-Iキャリアであることを知り，大きなショックを受ける．
2. 母子感染予防法があることを知り，子どもには感染させたくないと訴える．
3. 夫，家族に結果を知らせるべきか悩む．
4. 夫と相談し，母乳栄養法について決定する．
5. 自分自身のATL，HAMのことで不安になる．

※1～5の間，カウンセリングが必要なことがある．

次に，子どもにHTLV-Iを感染させることを減らす方法があると説明すると，ほぼすべてのキャリア妊婦は，自分の子どもには感染させたくないと訴える．このときは，分かりやすく人工乳，凍結母乳，3カ月までの短期母乳について説明する．

次に必ず，自分がHTLV-Iキャリアであることを夫や家族に伝えるべきか悩む．本人の悩みは深刻なので，医療者側からアドバイスをするとよい．夫婦の状況によって変わると思うが，可能であれば夫には相談したほうがよいと考える．その理由は，HTLV-Iは「親の意思」によって防ぐことが可能な感染症であり，子どもの将来を決定するためには，2人で相談したほうがよいからである．またキャリアである妊婦を支えてくれるのは夫であるからである．しかし，そのほかの家族にキャリアであることを伝える必要は原則的にはなく，またメリットも特別の場合を除いてない．

その後，夫と本人とに対して3つの授乳方法を説明し，いずれかの授乳方法を選択した際は，助産師・看護師は母乳外来などで対応することになる．人工乳を選択した場合は，目の前でほかの患者に乳房管理の指導を行うことは避けるなど，配慮していただきたい．場合によっては相談にも乗ってほしい．凍結母乳もしくは短期母乳を選択した場合は，積極的に母乳外来などで出産前の乳房管理に努めてほしい．凍結母乳を選択した場合は，搾乳の方法や母乳パックなどの情報を提供していただきたい．3カ月までの短期授乳を選択された場合，2カ月末から3カ月に入った時点で，徐々に粉ミルクに切り替えていく方法を提示していただきたい．

またいったん，授乳法を決めたが，その後に気持ちが揺らぐこともあるので，その際も時間を取って，本人が納得するまで十分に相談に乗ってあげてほしい．

最後にキャリア妊婦は「自分がこの先どうなるのか？」ということで必ず悩む．分娩後もしくは40歳以降になってから，2011年度に全国で設置される予定となっている「HTLV-Iキャリア外来」を紹介して，専門的な話をしてもらうことを勧めていただきたい．種々の不安を解消した上で，お産に臨み，その後の育児もスムーズにいくよう支援していただきたい．また出産後の赤ちゃんは小児科でフォローしてもらい，3歳時の採血で感染の有無が分かることも説明してほしい．小児科外来でも看護師は育児に関する種々の悩みや質問を受けると思うので，その際も丁寧な説明をお願いしたい．

おわりに

国が本格的にHTLV-I撲滅に向けて動き出した．この中で母子感染予防対策は，最重要課題である．全国で毎年約3,000人の妊婦が突然，HTLV-Iキャリアであると告げられることになるが，十分な説明の上で，医師，助産師，看護師が協力し合って，HTLV-I母子感染が減少し，かつキャリアの健康が維持されることを望む．

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Original article

Gag-CA Q110D mutation elicits TRIM5-independent enhancement of HIV-1mt replication in macaque cells

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Abstract

HIV-1 is strictly adapted to humans, and cause disease-inducing persistent infection only in humans. We have generated a series of macaque-tropic HIV-1 (HIV-1mt) to establish non-human primate models for basic and clinical studies. HIV-1mt clones available to date grow poorly in macaque cells relative to SIVmac239. In this study, viral adaptive mutation in macaque cells, G114E in capsid (CA) helix 6 of HIV-1mt, that enhances viral replication was identified. Computer-assisted structural analysis predicted that another Q110D mutation in CA helix 6 would also increase viral growth potential. A new proviral construct MN4Rh-3 carrying CA-Q110D exhibited exquisitely enhanced growth property specifically in macaque cells. Susceptibility of MN4Rh-3 to macaque TRIM5α/TRIMCyp proteins was examined by their expression systems. HIV-1mt clones so far constructed already completely evaded TRIMCyp restriction, and further enhancement of TRIMCyp resistance by Q110D was not observed. In addition, Q110D did not contribute to evasion from TRIM5α restriction. However, the single-cycle infectivity of MN4Rh-3 in macaque cells was enhanced relative to the other HIV-1mt clones. Our results here indicate that CA-Q110D accelerates viral growth in macaque cells irrelevant to TRIM5 proteins restriction.

Keywords: HIV-1; HIV-1mt; Gag-CA; Macaque cells; Virus growth; Molecular modeling

1. Introduction

Mammalian cells express a variety of host restriction factors to defend themselves against pathogens. Viruses have evolved countermeasures to subvert their restriction and replicate efficiently in cells [1,2]. HIV-1, a causative agent of human AIDS, evades host restriction factors and replicates well in human cells. However, in macaques for experimental use, e.g. cynomolgus macaques (CyMs) and rhesus macaques (RhMs), HIV-1 replication is completely inhibited by host restriction factors present in their cells [3]. Construction of HIV-1 that overcomes species-barrier contributes much to understand the interaction of HIV-1 and its host as well as the establishment of HIV-1-infected macaque models [4,5].

Extensive molecular biological studies on the HIV-1/host interaction conducted to date have revealed main mechanical bases for the narrow host range exhibited by HIV-1. Macaque cells contain potent antiviral factors that effectively restrict or even abolish HIV-1 replication. These include APOBEC3 proteins (APOs), CyclophilinA (CypA), and TRIM5α/TRIMCyp

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(TRIM5 proteins). HIV-1 can indeed counteract human proteins corresponding to these restriction factors. APOs exhibit cytidine deaminase activity, and introduce lethal mutations into HIV-1 genome. HIV-1 Vif is able to neutralize the antiviral activity of human APOs, but not macaque APOs [6–8]. CypA acts on incoming HIV-1 core to regulate infection positively in human cells but negatively in macaque cells [9–11], though amino acid sequences of CypA from human and macaque are identical. Macaque TRIM5α recognizes and interacts with incoming HIV-1 core, and restricts virus infection in a less-defined mechanism [9–11]. Macaque TRIM5α is polymorphic, and has sequence variation in a C-terminal B30.2/SPRY domain important for capsid (CA) binding. Sequence variation in this domain causes modulation of host susceptibility to retrovirus infection [12,13]. Macaque TRIMCyp is a fusion protein resulted from replacement of a B30.2/SPRY domain with CypA. Both CyM and RhM cells express TRIMCyp, but affinity of these proteins to HIV-1 core is different due to amino acid substitutions in Cyp domains. Thus, CyM TRIMCyp restricts HIV-1 replication, but not RhM TRIMCyp [14,15].

Identification of host restriction factors in macaque cells and their target proteins in HIV-1 has prompted us to generate macaque-tropic HIV-1 (HIV-1mt) with a minimal modification of HIV-1 genome. We successfully constructed prototype HIV-1mt, NL-DT5R, by replacing CypA binding region on a loop between helices 4 and 5 (h4/5L) in *gag*-CA and entire *vif* genes with the corresponding regions of pathogenic SIVmac239 (Fig. 1) [16]. But growth potential of NL-DT5R was inferior to that of SIVmac239 both *in vitro* and *in vivo* [16,17]. These results indicated that genetic modifications in NL-DT5R were insufficient to confer the ability on the virus to grow efficiently in macaque cells [16–18]. In an attempt to improve growth potential of NL-DT5R, we adapted NL-DT5R and its R5-tropic version NL-DT562 to a CyM derived lymphocyte cell line HSC-F, and found a number of genetic substitutions in viral genomes of adapted viruses [19]. We introduced these mutations and CA h6/7L from SIVmac239 into NL-DT5R, and the resultant clone was designated MN4-5S (Fig. 1) [19]. MN4-5S exhibited enhanced growth potential in CyM both *in vitro* and *in vivo* compared to NL-DT5R [19]. But growth ability of MN4-5S was still lower than that of SIVmac239.

In this study, to further improve replication potential of HIV-1mt, we adapted MN4-5S in macaque cells and identified an adaptive mutation in CA that enhances growth ability in the cells. *In silico* structural modeling of the adaptive mutation predicted that Q110D mutation on helix 6 in CA (CA-Q110D) would promote viral replication in macaque cells. Indeed, a proviral clone carrying CA-Q110D, designated MN4Rh-3, exhibited marked enhancement of growth potential in macaque cells relative to all the other HIV-1mt clones we have constructed (Fig. 1). CyM TRIM5α/TRIMCyp susceptibility assays revealed that MN4Rh-3 completely evades from TRIMCyp restriction but not TRIM5α restriction as observed for the other HIV-1mt clones. While CA-Q110D contributed to neither endowment of further resistance to TRIMCyp nor evasion from TRIM5α restriction, CA-Q110D did lead to enhanced single-cycle infectivity to a macaque cell line compared with the other HIV-1mt clones. Our results here indicate that CA-Q110D accelerates viral growth in macaque cells independently of TRIM5 proteins restriction.

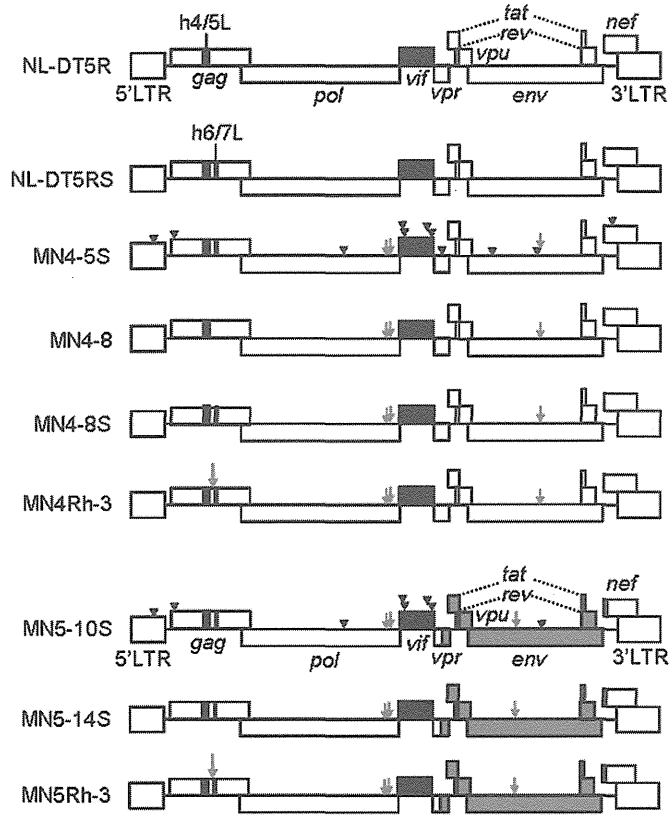


Fig. 1. Proviral genome structure of various HIV-1mt clones used in this study. HIV-1 NL4-3 [26] and SIVmac239 (GenBank: M33262) sequences are indicated by white and black areas, respectively. Gray areas in MN5-10S, MN5-14S and MN5Rh-3 show sequences from NF462 [21]. Blue arrows and black arrowheads show nucleotide substitutions that appeared in viral genomes of NL-DT5R and NL-DT562 during adaptation in HSC-F cells. Among nucleotide substitutions, adaptive mutations that enhance viral growth potential are indicated by blue arrows. Red arrows show the CA-Q110D mutation.

2. Materials and methods

2.1. Plasmid DNA

Construction of NL-DT5R, NL-DT562, NL-DT5RS, and MN4-5S were described previously [16,19–21]. MN4-5S carries all nucleotide substitutions that are present in adapted NL-DT5R and NL-DT562 clones except for mutations in the *env* gene of R5-tropic viruses (MN5-10S, MN5-14S, and MN5Rh-3 in Fig. 1) [19]. MN4-8S contains adaptive (growth-enhancing) mutations in MN4-5S but not the other mutations. MN4Rh-3 was constructed by introduction of the CA-Q110D mutation into MN4-8S. To construct R5-tropic viruses, 3′ halves of viral genomes (*Eco*RI in *vpr* to *Sph*I at the 3′ end of viral genome) of MN4-5S, MN4-8S, and MN4Rh-3 were replaced with the corresponding regions of adapted-NL-DT562,

and were designated MN5-10S, MN5-14S, and MN5Rh-3, respectively. For single-cycle infectivity assays to monitor viral susceptibility to TRIM5 proteins and to determine infectivity for CyM cells, *env*-deficient HIV-1mt variants encoding luciferase gene were constructed. NL-DT5R was cleaved with *Nde*I and *Nhe*I (both sites in *env* gene), blunt ended by T4 DNA polymerase, and resealed by T4 DNA ligase. The resultant clone was designated 5RΔEnv. Luciferase gene was then introduced into *nef* gene of 5RΔEnv as described previously [22], and the resultant clone was designated 5RΔEnv + Luc. A fragment containing the 3′ half genome was cut out from the 5RΔEnv + Luc, and introduced into the corresponding region in HIV-1mt variants (DT5R/4-3, NL-DT5RS, MN4-8, MN4-8S, and MN4Rh-3) to generate 5R/4-3ΔEnv + Luc, 5RSΔEnv + Luc, 4-8ΔEnv + Luc, 4-8SΔEnv + Luc, and 4Rh-3ΔEnv + Luc, respectively.

2.2. Cell culture

A human monolayer cell line 293T [23], a feline kidney cell line CRFK (ATCC CCL-94), and a CyM kidney cell line MK.P3(F) (JCRB 0607) were maintained in Eagles's minimal essential medium (MEM) containing 10% heat-inactivated fetal bovine serum (hiFBS). CRFK cells expressing TRIM5α/TRIMCyp were maintained in MEM containing 10% hiFBS and 400 μg/mL G418 (SIGMA). Macaque lymphocyte cell lines, HSC-F [24] and HSR5.4 [25], were maintained in RPMI-1640 medium containing 10% hiFBS. Recombinant human IL-2 (AbD Serotec) was added to the medium (50 units/mL) for maintenance of HSR5.4 cells. A human lymphocyte cell line MT4/CCR5 (MT4 cells stably expressing CCR5) was maintained in RPMI-1640 medium containing 10% hiFBS and 200 μg/mL hyglomycine (SIGMA).

2.3. Virus replication assays

Virus stocks for infection were prepared from 293T cells transfected with proviral clones as described previously [16,19,26]. Virion-associated reverse transcriptase (RT) activity was measured as described previously [16]. HSC-F cells (10^6) were infected with equal RT units of viruses in the presence of IL-2. For infection of MT4/CCR5 cells (10^6), the spinoculation method [27] was used. Viral growth was monitored by RT activity released into the culture supernatants. We assessed the viral growth potential by the peak day of virus production, and if the viral growth kinetics are similar, by the production level on the peak day.

2.4. Generation and characterization of adapted viral clones

MN4-5S and MN5-10S viruses (Fig. 1) prepared from transfected 293T cells were inoculated into HSR5.4 cells (3×10^6) with an equal amount of viruses (5×10^7 RT units). The cultures were maintained in the presence of IL-2, and HSC-F cells were added on day 34 post-infection. The culture supernatants (collected on day 18 post-cocultivation, the peak day of virus production) were inoculated into fresh HSR5.4 cells, and total DNA was extracted from the cells on day 15 post-infection. Integrated proviruses were amplified from total DNA as two overlapping fragments by the polymerase chain reaction (PCR), and amplified products were cloned into MN5-10S as described previously [16]. Viruses were prepared from 293T cells transfected with the resultant clones, and inoculated into HSR5.4 cells. Only one clone exhibited a rapid growth kinetics compared to MN5-10S, and was designated Ad clone-25. To identify an adaptive mutation that enhances growth potential, each mutation found in the genome of Ad clone-25 was introduced into MN5-14S by site-directed mutagenesis (STRATAGENE). For screening, viruses prepared from transfected 293T cells were inoculated into HSC-F cells, and virus replication was monitored by RT activity released into the culture supernatants.

2.5. Molecular modeling of HIV CA N-terminal domain (NTD)

The crystal structure of HIV-1 CA NTD at a resolution of 2.00 Å (PDB code: 1M9C [28]) was taken from the RCSB Protein Data Bank [29]. The three-dimensional (3-D) models of HIV-1 CA NTD were constructed by the homology modeling technique using 'MOE-Align' and 'MOE-Homology' in the Molecular Operating Environment (MOE) (Chemical Computing Group Inc., Quebec, Canada) as described [30–32]. We obtained 25 intermediate models per one homology modeling in MOE, and selected the 3-D models which were the intermediate models with best scores according to the generalized Born/volume integral methodology [33]. The final 3-D models were thermodynamically optimized by energy minimization using an AMBER99 force field [34] combined with the generalized Born model of aqueous solvation implemented in MOE [35]. Physically unacceptable local structures of the optimized 3-D models were further refined on the basis of evaluation by the Ramachandran plot using MOE.

2.6. Single-cycle infectivity assays

To generate CRFK cells expressing CyM TRIMCyp, the cDNA was isolated from HSC-F cells, and expression vector of FLAG-tagged CyM TRIMCyp was constructed as described previously [18]. The sequence of TRIMCyp from HSC-F cells was identical with Mafa TRIMCyp2 (GenBank: FJ609415). CRFK cell lines expressing CyM TRIMCyp were selected by G418 as described previously [18]. Expression and inhibitory effect of the selected cell clones were verified by Western blotting with anti-FLAG antibody (SIGMA) and by infection with vesicular stomatitis virus G protein (VSV-G) pseudotyped 5R/4-3ΔEnv + Luc, respectively. Assays using naïve CRFK, CRFK expressing CyM TRIM5α [18] or CyM TRIMCyp, and MK.P3(F) cells were similarly performed as described previously [36]. VSV-G pseudotyped virus stocks were prepared from 293T cells transfected with individual HIV-1mtΔEnv + Luc clones and pCMV-G (GenBank: AJ318514)

at a molar ratio of 1:1. Naïve CRFK, CRFK expressing TRIM5α/TRIMCyp and MK.P3(F) cells were infected with an equal titer of viruses (to generate approximately 10^7 relative luminescence (RLU) for naïve CRFK cells), and on day 2 post-infection, cells were analyzed for luciferase activity. Assays using recombinant Sendai virus (SeV)-CyM TRIM5α/TRIMCyp expression system were performed as described previously [31].

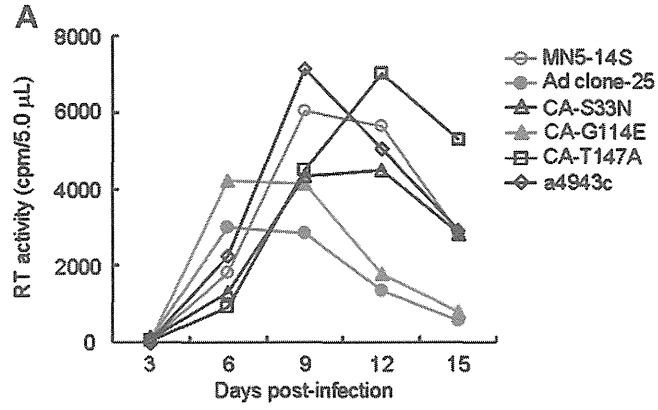
3. Results

3.1. An adaptive mutation G114E on helix 6 in CA (CA-G114E) enhances viral growth potential in macaque cells

An HIV-1mt variant MN4-5S replicated more slowly than SIVmac239 in macaque cells. In order to improve its growth potential, we carried out virus adaptation in a macaque lymphocyte cell line HSR5.4. Virus adaptation was performed by long-term culture of HSR5.4 cells infected with MN4-5S (X4-tropic) or its R5-tropic version MN5-10S (Fig. 1). Construction of proviral clones from adapted viruses was described in Materials and methods. We obtained only one clone (Ad clone-25) with enhanced growth potential from 100 proviral clones constructed and tested. We sequenced the entire genome of Ad clone-25, and found three non-synonymous mutations in CA (S33N, G114E, and T147A in Fig. 2A) and one synonymous mutation in integrase (IN)(a4943c in Fig. 2A). To identify an adaptive mutation that enhances growth potential, each mutation found in Ad clone-25 was introduced into a parental clone MN5-14S (Fig. 1). MN5-14S carries only growth-promoting mutations in MN5-10S, and the two clones exhibit similar growth potential in macaque cells. Viruses were prepared from 293T cells transfected with MN5-14S, Ad clone-25, or clones carrying individual mutations, and inoculated into HSC-F cells (Fig. 2A). Only one clone carrying CA-G114E exhibited similar growth kinetics to that of Ad clone-25 but not the others. This result indicates that CA-G114E is an adaptive mutation enhancing growth potential of HIV-1mt in macaque cells. This mutation is exactly the same as the previously found adaptive mutation, which enhanced growth of NL-4/5S6/7SvifS virus in human CEM-SS cells [37]. NL-4/5S6/7SvifS virus is a prototype HIV-1mt bearing the same CA with that of MN4-5S.

3.2. Molecular modeling of the CA NTD of HIV-1mt variants suggests that CA-G114E and CA-Q110D mutations have a similar positive effect on viral replication

The amino acid at position 114 is located in CA NTD. To obtain structural insights into impacts of the G114E substitution in order to improve growth capability of HIV-1mt variants in macaque cells, we conducted computer-assisted structural study: we constructed 3-D models of CA NTD of three HIV-1mt variants, CA-G114E, CA-G114Q, and MN4-5S, using homology-modeling technique (see Materials and methods). Main chain folds of the three models were indistinguishable, suggesting that 3-D position and type of side chain are critical



Nucleotide change	Region	Amino acid change in the region
g1283a	CA	S33N
g1526a	CA	G114E
a1624g	CA	T147A
a4943c	IN	None

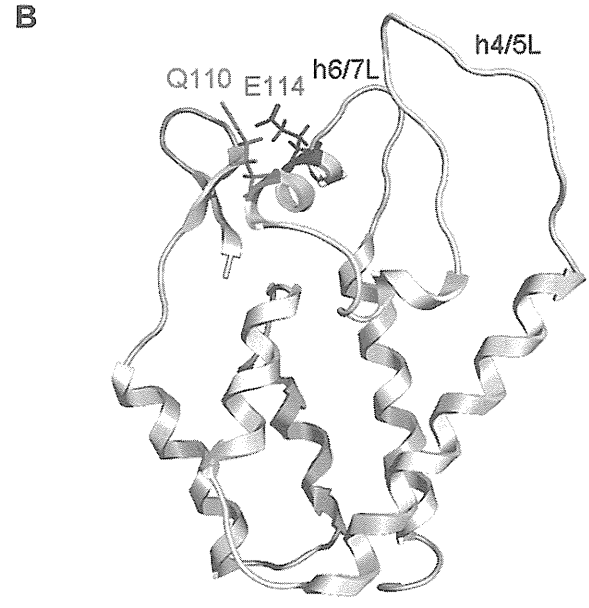


Fig. 2. Mutations in Gag-CA. (A) Identification of an adaptive mutation that enhances viral growth. Nucleotide substitutions found in the genome of Ad clone-25 are indicated at the bottom. Virus samples were prepared from 293T cells transfected with the indicated proviral clones, and equal RT units were inoculated into HSC-F cells. MN5-14S and Ad clone-25 served as controls. Virus replication was monitored by RT activity released into the culture supernatants. (B) 3-D structural models for CA NTD of HIV-1mt variants. Structural models of CA NTD of HIV-1mt variants were constructed by homology-modeling using "MOE-Align" and "MOE-Homology" in MOE as described previously [30–32]. Crystal structure of HIV-1 CA NTD at a resolution of 2.00 Å (PDB code: 1M9C [28]) was used as template for homology modeling. Main chain folds were indistinguishable among the models, and only the model of G114E CA is shown as a representative. Magenta and red sticks: side chains of 110th and 114th amino acid residues, respectively, of the G114E CA NTD.

for the phenotypic change. The modeling study revealed that 114th residue of G114E CA NTD is located on helix 6 in CA NTD such that its side chain protrudes into the exposed surface of CA (Fig. 2B). A charged amino acid residue on a protein surface participates in determining physicochemical properties of interaction surface of the protein and thus influences its structural and functional properties. Therefore, we assumed that the protrusion of a negatively charged side chain from helix 6 into exposed surface could have somehow a positive effect on growth capability of the HIV-1mt variants in macaque cells. In this regard, especially worth noting is that 110th amino acid residue on helix 6 of the HIV-1mt variant CAs was positioned on the same helical face with 114th amino acid residue (Fig. 2B). Therefore, we predicted that substitution of glutamine (Q) at position 110 by acidic amino acid such as aspartic acid (D) and glutamic acid (E) may also have a positive effect on growth capability of the HIV-1mt variants in macaque cells as G114E does. SIVmac239 has aspartic acid and glutamine at the positions 110 and 114, respectively.

3.3. CA-Q110D promotes viral growth more efficiently in macaque cells than CA-G114E mutation but its enhancing effect is species-specific

To confirm our prediction described above, CA-Q110D mutation was introduced into MN5-14S (designated MN5Rh-3), and the growth property in HSC-F cells of MN5Rh-3 and a viral clone carrying G114E (CA-G114E in Fig. 2A) was compared. As shown in Fig. 3A, MN5Rh-3 grew better than CA-G114E, indicating that CA-Q110D further accelerates HIV-1mt replication in macaque cells compared with an adaptive CA-G114E mutation. We next constructed an X4-tropic proviral clone carrying the CA-Q110D (designated MN4Rh-3) (Fig. 1), and compared its growth property with MN5Rh-3 in HSC-F cells (Fig. 3B). MN4Rh-3 was found to exhibit higher growth ability than MN5Rh-3, and was therefore used for infection experiments hereafter.

While CypA and TRIM5α have inhibitory effect on HIV-1 replication in macaque cells, CypA promotes HIV-1 infection in human cells and human TRIM5α only weakly inhibits HIV-1 replication [38–40]. Since the CA-Q110D mutation (acquisition of negatively charged side chain), as predicted by structural modeling, could impact on the interaction of HIV-1 CA and its binding factor(s) by altering physicochemical properties of CA binding surface, it can be speculated that CA-Q110D may promote viral replication specifically in macaque cells. Thus, we analyzed the effect of CA-Q110D on viral growth in macaque and human cells. In this experiment, we used HIV-1mt variants (MN4-8, MN4-8S, and MN4Rh-3) that have distinct CA structures (Fig. 1). Viruses prepared from transfected 293T cells were inoculated into macaque HSC-F and human MT4/CCR5 cells, and examined for growth property (Fig. 3C). The introduction of SIVmac239 CA h6/7L (MN4-8S) resulted in enhanced and reduced viral growth in macaque and human cells, respectively, relative to MN4-8. MN4Rh-3 grew clearly better in macaque cells relative to MN4-8 and MN4-8S, but more poorly in human cells than the other twos. These results demonstrate that the CA-Q110D mutation enhances viral replication in a host cell species-specific manner.

3.4. CA-Q110D does not contribute to evasion from CyM TRIM5 proteins restriction

We predicted that the growth enhancement by CA-Q110D may come from the increased resistance to CyM TRIM5 proteins, and therefore examined the susceptibility of HIV-1mt variants to them by two independent assays.

First, assays were performed in feline kidney CRFK cells expressing TRIM5α or TRIMCyp by using VSV-G pseudotyped viruses encoding the luciferase gene (Fig. 4A–C). The sequence differences between HIV-1mt variants reside only in CA and IN (Figs. 1 and 4). Since adaptive mutations in IN contribute to enhancement of virion production but not early replication phase (manuscript in preparation), only the difference in CA affects the relative single cycle infectivity in this assay. A pseudotyped virus 5R/4-3 carries HIV-1 (NL4-3) CA without any modifications and served as negative control. While 5R and 4-8 have an identical CA structure carrying h4/5L from SIVmac239, 5RS and 4-8S have both h4/5L and h6/7L from SIVmac239 CA. 4Rh-3 carries CA-Q110D mutation in addition to h4/5L and h6/7L from SIVmac239 CA. Viral infectivity was measured by luciferase activity in infected cells and presented as RLU. Naïve CRFK and CRFK cells expressing TRIM5α were infected with an equal amount of viruses generating 10^7 RLU in naïve cells. As shown in Fig. 4B, the infectivity of 5R and 4-8 for cells expressing CyM TRIM5α was similar to that of a negative control 5R/4-3. However, higher infectivity was observed for 5RS and 4-8S relative to 5R and 4-8. These results were consistent with previous reports that h4/5L and h6/7L in HIV-1 CA are a part of determinant for TRIM5α restriction [20,36]. The sensitivity of 4Rh-3 to TRIM5α was similar to that of 5RS and 4-8S. This indicates that CA-Q110D did not contribute to increase the resistance to TRIM5α. It has been reported that CyM TRIMCyp has the ability to restrict HIV-1 replication [15]. To examine the susceptibility of HIV-1mt variants to TRIMCyp, we generated feline CRFK cells expressing TRIMCyp, and the cells were infected with pseudotyped viruses as described above. As shown in Fig. 4C, all the clones tested were more resistant to a similar extent to TRIMCyp than the control 5R/4-3. In agreement with a previous study showing that elimination of alanine at position 88 within h4/5L of HIV-1 CA confers the resistance on the virus to TRIMCyp [15], our results indicate that the replacement of HIV-1 CA h4/5L with that of SIVmac239 is sufficient for HIV-1mt to evade from the TRIMCyp restriction. Second, we performed another susceptibility assay using the recombinant SeV expression system. This system assures a very high expression level of target proteins in cells infected with the recombinant SeV. Therefore, the ability of viruses to completely counteract the restriction effect of TRIM5 proteins could be determined by MT4/SeV-TRIM5 expression system. Human MT4 cells were infected with recombinant SeV expressing CyM TRIM5α, TRIMCyp, or SPRY(−)TRIM5, and then super-infected with HIV-1

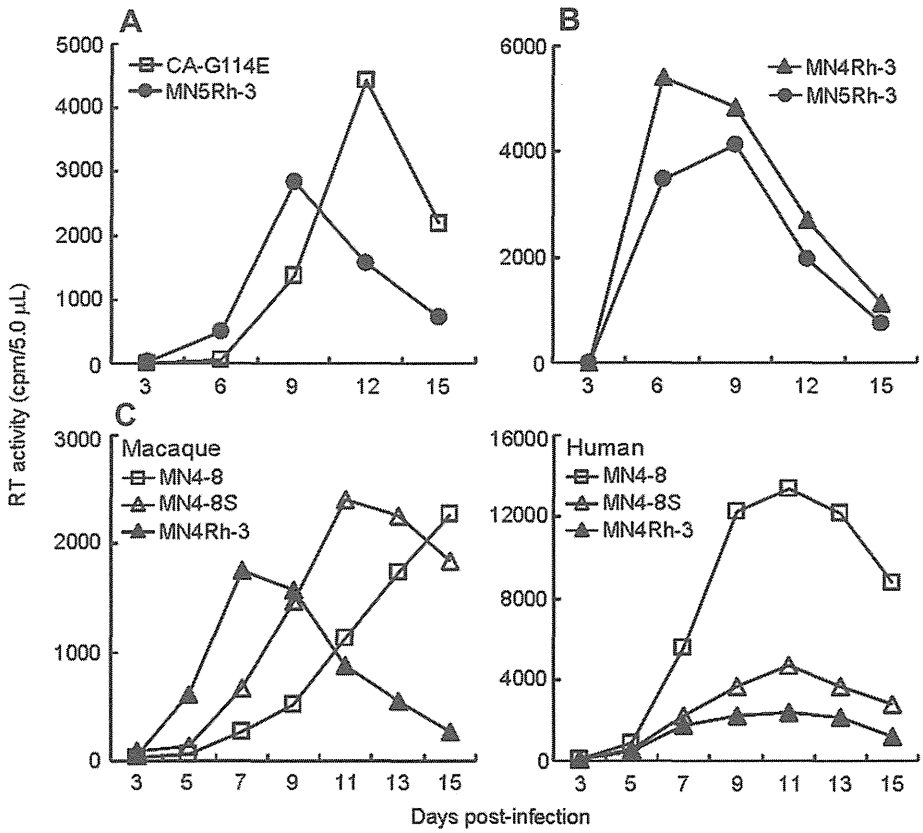


Fig. 3. Effect of CA modification on viral growth in macaque and human lymphocyte cell lines. (A and B) Growth kinetics of HIV-1mt clones carrying CA-G114E or CA-Q110D (MN5Rh-3 and MN4Rh-3) in CyM HSC-F cells. Virus samples were prepared from 293T cells transfected with the indicated proviral clones, and equal amounts (5×10^5 RT units) were inoculated into HSC-F cells (10^6). Virus replication was monitored by RT activity released into the culture supernatants. (C) Growth kinetics of MN4-8, MN4-8S, and MN4Rh-3 in HSC-F (Macaque) and MT4/CCR5 (Human) cells. Virus samples were prepared from 293T cells transfected with the indicated proviral clones, and equal amounts (10^6 RT units) were inoculated into HSC-F cells (10^6). For spinoculation of MT4/CCR5 cells (10^6), 6×10^5 RT units were used as inocula. Virus replication was monitored by RT activity released into the culture supernatants.

(NL4-3), SIVmac239, or HIV-1mt variants. SPRY(−)TRIM5 which can not bind to viral CA served as control. NL4-3 and SIVmac239 also served as controls for viral replication. As shown in Fig. 4D, NL4-3 replicated in cells expressing SPRY(−)TRIM5, but not in TRIM5α and TRIMCyp expressing cells. SIVmac239 exhibited similar growth kinetics in SPRY(−)TRIM5, TRIM5α and TRIMCyp expressing cells. All HIV-1mt variants replicated in TRIMCyp expressing cells similarly well in SPRY(−)TRIM5 cells. Together with assays in CRFK cells, these results showed that all HIV-1mt variants except for 5R/4-3 completely evade from TRIMCyp restriction. In contrast, the growth of all HIV-1mt variants was inhibited in CyM TRIM5α expressing MT4 cells. These results indicate that HIV-1mt variants do not evade from TRIM5α restriction as effectively as SIVmac239.

Results obtained by our two assay systems with respect to the susceptibility of HIV-1mt variants to CyM TRIM5α were apparently different (Fig. 4B and D), but this difference is most likely to be due to the TRIM5α expression level. In MT4 cells infected with recombinant SeV, TRIM5α is expressed at much higher level than that in transduced CRFK cells, masking the increase of resistance to TRIM5α detectable by the transduced CRFK system (Fig. 4B). Indeed, the growth enhancement of 5RS relative to 5R [20] can be explained by the results in Fig. 4B but not those in Fig. 4D. The apparent discrepancy of the sensitivity depending on TRIM5α expression level was also observed between B-LCL cells and transduced CRFK cells [41]. In sum, we can conclude here that MN4Rh-3 exhibits a partial resistance to TRIM5α insufficient for complete evasion as 5RS and 4-8S do, and that the CA-Q110D mutation is irrelevant to this property.

3.5. CA-Q110D enhances viral infectivity for macaque cells

Results so far showed that CA-Q110D does not contribute to evasion from TRIM5 proteins restriction in rather artificial systems using feline and human cells (Fig. 4). To investigate further how CA-Q110D enhances viral replication, we examined single-cycle viral infectivity in macaque cells. CyM kidney MK.P3(F) cells, which have heterozygote for TRIM5α and TRIMCyp, were infected with various VSV-G pseudoviruses and analyzed for their infectivity as described above. As shown in Fig. 5A, viral infectivity was increased by modification of h4/5L (compare 5R/4-3 and 5R&4-8). Modification of h6/7L in addition to h4/5L further augmented viral infectivity (compare 5R&4-8 and 5RS&4-8S). Introduction of the CA-Q110D mutation into 4-8S clone gave the highest infectivity among the viruses tested (see 4Rh-3). The results in

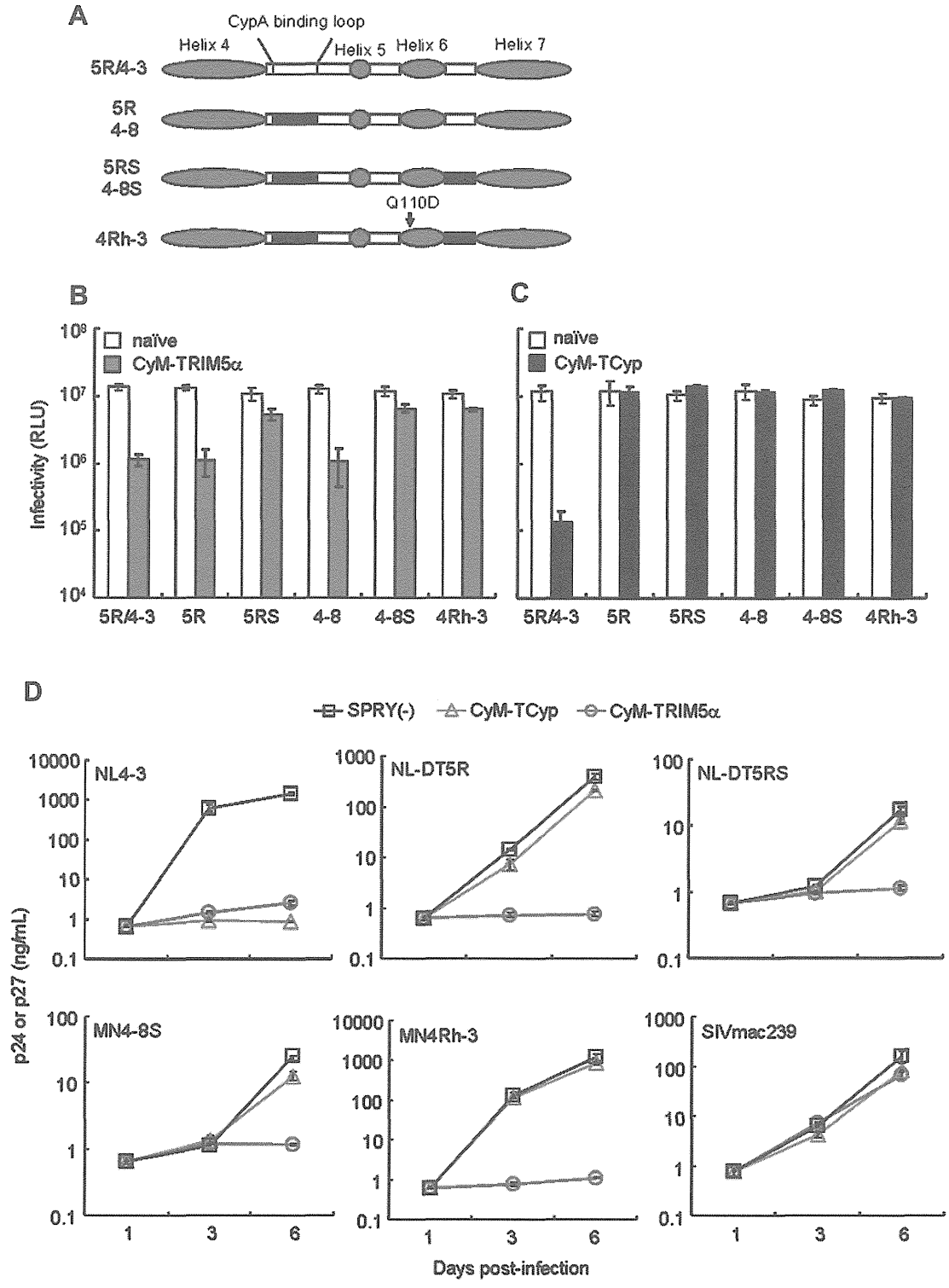


Fig. 4. Effect of CA modification in HIV-1mt variants on viral infectivity. (A) CA structure of viral clones used in TRIM5α/TRIMCyp susceptibility assays. Blue and white areas show helices and loops from HIV-1 NL4-3 CA, respectively. Sequences from SIVmac239 are indicated by black areas. (B and C) Susceptibility of HIV-1mt variants to CyM TRIM5 proteins as examined by CRFK system. Results for CyM TRIM5α (B) and for CyM TRIMCyp (TCyp) (C) are shown. VSV-G pseudotyped viruses were prepared from transfected 293T cells as input samples. Viruses generating 10^7 RLU in CRFK-naïve cells were inoculated into CRFK cells that express CyM TRIM5α or CyM TCyp. On day 2 post-infection, cells were analyzed for luciferase activity by a luminometer. (D) Susceptibility of HIV-1mt variants to CyM TRIM5 proteins as examined by SeV system. Human MT4 cells (10^5) were infected with recombinant SeV expressing CyM TRIM5α, TRIMCyp, or SPRY (−) TRIM5. Nine hours after infection, cells were super-infected with 20 ng (Gag-p24) of HIV-1 NL4-3, various HIV-1mt clones, or 20 ng (Gag-p27) of SIVmac239. Virus replication was monitored by the amount of Gag-p24 from NL4-3 and HIV-1mt clones or Gag-p27 from SIVmac239 in the culture supernatants. Error bars show actual fluctuations between duplicate samples. Data from one representative of three independent experiments are shown.

Fig. 5A show that CA-Q110D uniquely increases viral infectivity in macaque cells not observed in the other experimental systems (Fig. 4), and suggest that some factor(s) in CyM cells other than TRIM5α and TRIMCyp proteins is associated with this enhancement.

As shown in Fig. 5B, MN4Rh-3 displayed slower growth kinetics relative to those of SIVmac239 (note the peak day of virus production), although it grew better than the other HIV-1mt clones in CyM HSC-F cells. Approximately 100-fold more input virus (RT units) compared to SIVmac239 was required for MN4Rh-3 to exhibit similar growth kinetics with SIVmac239 (data not shown). These results have shown that even MN4Rh-3 grows more poorly in macaque cells than a standard SIVmac clone pathogenic for macaque monkeys.

4. Discussion

In this study, we have demonstrated that a single CA mutation (Q110D) greatly promotes HIV-1mt growth in macaque cells (Fig. 3). This enhancing effect was afforded independently of TRIM5 proteins restriction. The virus carrying the CA-Q110D mutation (MN4Rh-3) certainly overcame the anti-viral action of CyM TRIMCyp but not completely CyM TRIM5α. However, the mutation itself (Fig. 1) did not influence anti-TRIMCyp/TRIM5α activity of MN4Rh-3 reported here (Fig. 4). Notably, this mutation exquisitely enhanced viral growth in macaque cells (Fig. 3) by augmenting viral single-cycle infectivity (Fig. 5). The viral growth enhancement reported here is well reproduced in CyM peripheral blood mononuclear cells and in CyMs (manuscript in preparation).

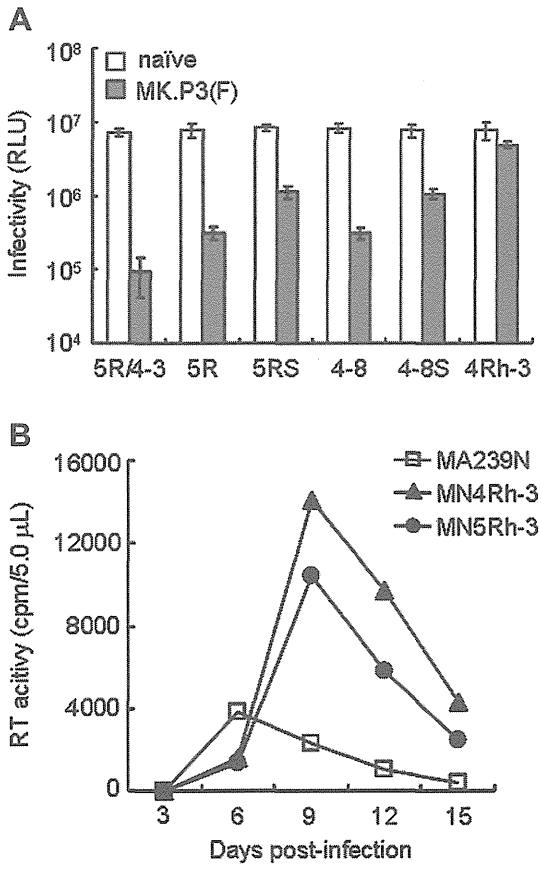
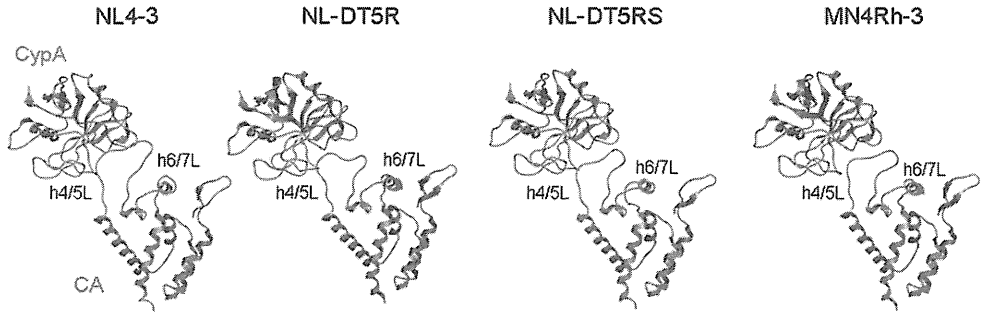


Fig. 5. Replication ability of various viruses in CyM cells. (A) Single-cycle infectivity of various HIV-1mt clones in CyM kidney MK.P3(F) cells. VSV-G pseudotyped viruses indicated were prepared from transfected 293T cells. MK.P3(F) cells were infected with an equal titer of viruses giving 10^7 RLU in CRFK-naïve cells. On day 2 post-infection, cells were analyzed for luciferase activity by a luminometer. (B) Multi-cycle growth kinetics of SIVmac and HIV-1mt viruses in CyM lymphocyte HSC-F cells. Virus samples were prepared from 293T cells transfected with the indicated proviral clones, and equal amounts (10^4 RT units) were inoculated into HSC-F cells (10^6). Virus replication was monitored by RT activity released into the culture supernatants. MA239N, an infectious clone of SIVmac239 with *nef*-open.

Regarding the mechanism for enhancement of viral growth by CA-Q110D, we initially thought a possibility that CA-Q110D compensates the disadvantage in HIV-1mt genome resulted from replacement of HIV-1 CA h4/5L and h6/7L with those of SIVmac239. However, this is highly unlikely because the enhancing effect is macaque cell-dependent (Fig. 3). Most feasible explanation is that CA-Q110D contributes to evade from a negative factor(s) in macaque cells such as CypA. Because HIV-1mt CA was designed not to bind to CypA, and the interaction between the two molecules was indeed undetectable by monitoring CypA virion-incorporation [18,20], we analyzed the binding by computer-assisted structural modeling. Homology modeling of the CA-CypA complexes was performed based on the crystal structure of HIV-1 CA NTD bound to CypA (PDB code: 1M9C [28]), and the binding energies, E_{bind}, were calculated using MOE as described previously [42,43]. As shown in Fig. 6, HIV-1 (NL4-3) CA was predicted to interact with CypA via its h4/5L (binding energy: −64.4 kcal/mol). The binding energy of CA and CypA was decreased by CA modifications, such as h4/5L replacement (NL-DT5R: −31.0 kcal/mol), h4/5L and h6/7L replacement (NL-DT5RS: −36.1 kcal/mol), and Q110D substitution in addition to h4/5L and h6/7L replacement (MN4Rh-3: −30.1 kcal/mol). Decrease in E_{bind} in NL-DT5R is consistent with the result that the h4/5L region directly interacts with CypA [28]. Notably, the E_{bind} for the NL-DT5RS CA was greater than that of the NL-DT5R and MN4Rh-3 CAs. These results suggest that not only h6/7L replacement but also Q110D substitution can influence structure of CypA binding surface of CA. The Q110D substitution is located on the exposed surface of helix 6 connecting to the h6/7L (Fig. 2B). CA helix 6 has been reported to interact with CypA binding region on h4/5L through hydrogen bonding [44,45]. Thereby it is reasonable that the local electrostatic change on the helix 6 by the Q110D substitution influenced structures of h4/5L via changes in fluctuation and conformation of h6/7L. This in turn could lead to reduction in stability of the MN4Rh-3 CA-CypA complex compared with NL-DT5RS CA-CypA complex, as predicted in Fig. 6. Our computer-assisted structural study suggests that the Q110D substitution can induce electrostatic modulation of the overall CA surface structure including h4/5L and h6/7L. Similar modulation mechanism of binding surface structures via charged amino acid substitution at distant site from the binding surface has been reported for Cyp domain of CyM TRIMCyp [15] and CD4 binding site of HIV-1 gp120 outer



	NL4-3	NL-DT5R	NL-DT5RS	MN4Rh-3
CA	HIV-1 CA	h4/5L from SIVmac239	h4/5L and h6/7L from SIVmac239	h4/5L and h6/7L from SIVmac239 and Q110D
Binding energy (kcal/mol)	-64.447	-31.012	-36.071	-30.051

Fig. 6. Structural models of HIV CA NTD bound to CypA. The model of CA NTD bound to CypA was constructed by homology modeling using the crystal structure of HIV-1 CA NTD and CypA complex (PDB code: 1M9C [28]). The binding energies, E_{bind} (kcal/mol), of the complex were calculated using MOE as described previously [42,43]. The formula $E_{bind} = E_{complex} - (E_{CA} + E_{CypA})$ was used for the E_{bind} calculation, where $E_{complex}$ is the energy of the CA/CypA complex models, E_{CA} is the energy of the CA monomer model, and E_{CypA} is the energy of the CypA monomer model.

domain [46]. Thus, it is not unreasonable to assume that the replication of MN4Rh-3 carrying CA-Q110D is enhanced in macaque cells but reduced in human cells by augmenting its dissociation from CypA (Fig. 6). However, it was found to be difficult to experimentally confirm this structural insight by determining the effect of cyclosporine A or of siRNA against CypA on viral infectivity because interaction between the HIV-1mt CA and CypA was so weak. Alternatively, CA-Q110D may contribute to the alteration of the affinity to unknown anti-CA factor(s) other than CypA and TRIM5 proteins. In this case, it is speculated that the factor(s) might act negatively on HIV-1 replication in macaque cells but positively in human cells, and vice versa. Further study is required to elucidate the mechanism for enhancement of viral growth potential by CA-Q110D.

In conclusion, further modification of the HIV-1mt genome is necessary to overcome unconquered replication block(s) present in macaque cells and obtain viral clones similarly replication-competent in macaque cells and pathogenic for animals with SIVmac (Fig. 5). Considering the genome structure of MN4Rh-3 and the results presented here, major targets for modification now are *gag*-CA (against TRIM5α) and *vpu* (against tetherin). Gag-CA is one of the two principal viral determinants (CA and Vif) for the HIV-1 species-tropism. Construction of HIV-1 CA that evades from TRIM5α restriction is also useful for elucidation of the less-defined CA-TRIM5α interaction and antiviral mechanism of TRIM5α. Tetherin, identified as anti-virion release factor, is antagonized by Vpu [47,48], but macaque tetherin was not counteracted by HIV-1 Vpu [49]. Construction of HIV-1 Vpu that down-modulate macaque tetherin may enhance viral replication *in vivo* as well as *in vitro* [50]. Through these approaches, we may be able to precisely analyze HIV-1 replication and pathogenesis *in vivo* and provide new strategies against HIV-1/AIDS.

Acknowledgments

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REVIEW ARTICLE
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Species tropism of HIV-1 modulated by viral accessory proteins

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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-capsid 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 evolutional 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

INTRODUCTION

Human immunodeficiency virus type 1 (HIV-1) is strictly adapted to humans, and cause disease-inducing persistent infection only in humans (Nomaguchi et al., 2008). This property is unique among primate immunodeficiency viruses, and represent one of the most evident and important viral characteristics to understand the biology/molecular biology of HIV-1. Of numerous primate immunodeficiency viruses so far identified (Kirchhoff, 2009; Sharp and Hahn, 2011), HIV-1 with an extremely limited host range exhibits exceptionally high replication ability, transmissibility, and pathogenicity in sensitive host humans. For basic HIV-1 researchers, it would be final goal to realize the basis/mechanism underlying these properties by experimental approaches.

Primate immunodeficiency viruses can be divided into three groups based on their genome structure in the central regions (Kirchhoff, 2009; Fujita et al., 2010; Sharp and Hahn, 2011). While viruses of HIV-1 type contain *vpr* and *vpu* genes, viruses of HIV-2 type carry *vpx* and *vpr* genes in tandem (**Figure 1**). The other simian immunodeficiency viruses (SIVs), the prototype virus, have only the *vpr* gene in the corresponding genomic region. HIV-1 is believed to emerge from the prototype virus via SIVmon/mus/gsn (isolated from the mona, mustached, and greater spot-nosed monkeys), SIVcpz (isolated from the chimpanzees), and SIVgor (isolated from the gorilla) through mutational and recombinational events. SIVmon/mus/gsn is known to recombine with SIVrcm (isolated from the red-capped mangabey monkey) to generate SIVcpz (for genome structures, see, **Figure 1**). SIVcpz served as parental virus for HIV-1 (M and N) and SIVgor (and finally for HIV-1 P).

The biological and molecular biological bases for species tropism of HIV-1 should reside in the above outlined evolutional 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.

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; Dorfman and Gottlinger, 1996), that Gag-capsid (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).

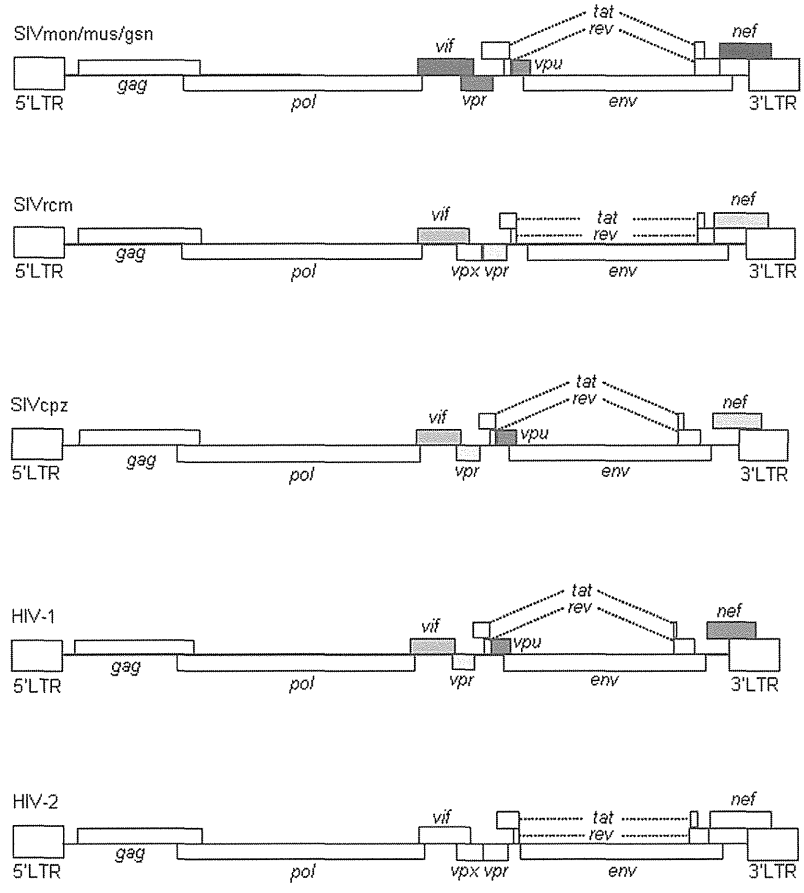

FIGURE 1 | Genome organization of primate immunodeficiency viruses. Various proviral genomes are schematically shown. As indicated by colored boxes, the *vpr* and *vpu* genes of SIVcpz/HIV-1 came from those of SIVrcm and SIVmon/mus/gsn, respectively. Also, the *vif* genes of SIVcpz/HIV-1 originated from 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	
Gag-CA	TRIM5α	Uncoating (early phase)
Gag-CA	TRIMCyp	Uncoating (early phase)
Vif	APOBEC3G	Reverse transcription (early phase)
	APOBEC3F	Reverse transcription (early phase)
Vpu	Tetherin/BST-2	Virion release (late phase)

For details, see references (Nakayama 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, unfairly 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

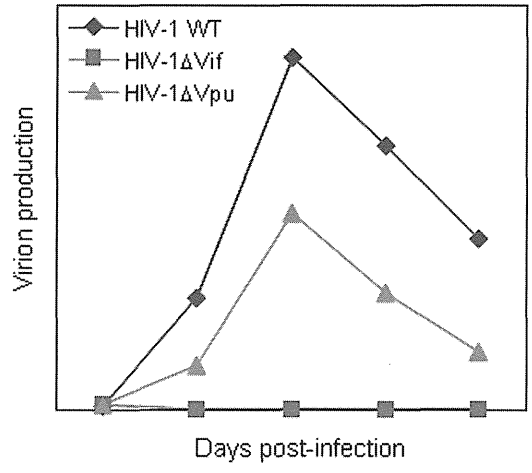

FIGURE 2 | A schema of replication kinetics by HIV-1 wild-type and mutant viruses. Viral growth properties in cells are illustrated based on numerous infection experiments in our laboratory. WT, wild-type.

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/BST-2. 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.

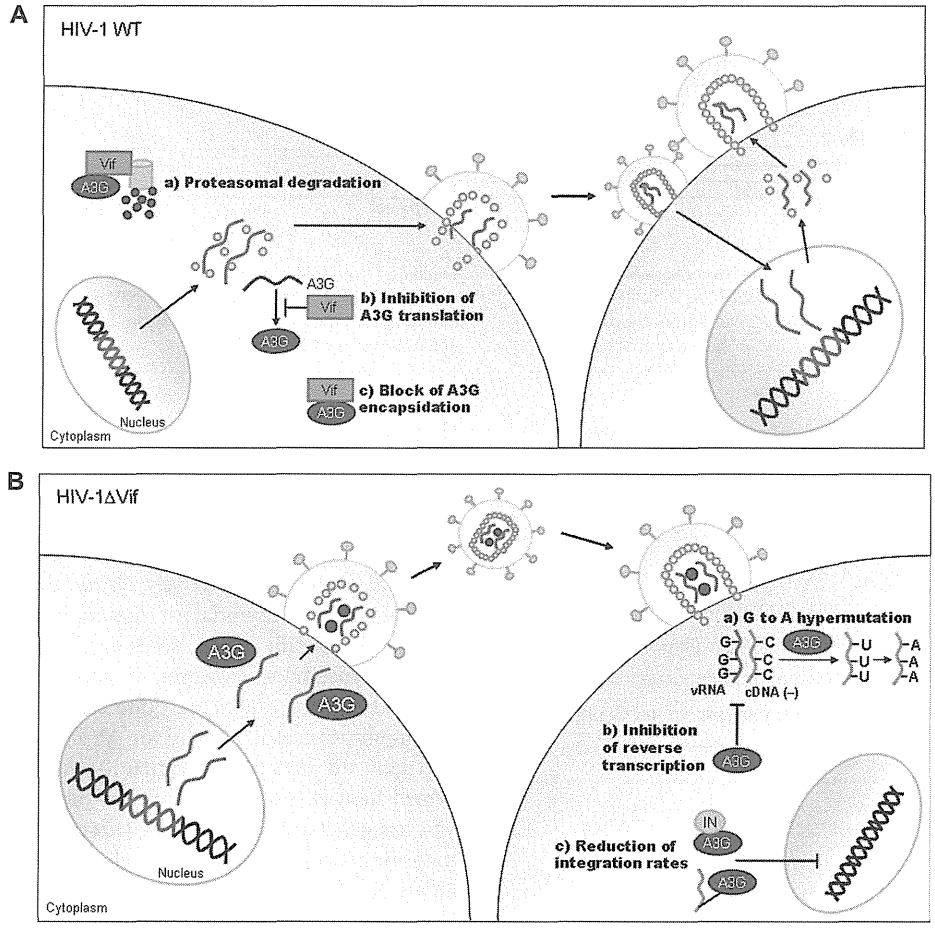

FIGURE 3 | HIV-1 replication and APOBEC3G. On the basis of results reported so far, the action mechanism of Vif is depicted. Replication process for wild-type (WT) and ΔVif mutant viruses are schematically shown on the basis of previously reported review articles (Holmes et al., 2007; Huthoff and Towers,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