厚生労働科学研究費補助金
エイズ対策研究事業
（H22-エイズ-若手-007）

霊長類ゲノム情報を利用した抗エイズウイルス自然免疫因子の探索およびその新規エイズ治療法への応用

平成２４年度　総括研究報告書

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目次

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研究要旨　世界的に流行しているエイズの原因であるヒト免疫不全ウイルス（HIV）は、サル免疫不全ウイルス（SIV）が「種の壁」を乗り越え病原性を示すHIVへと変貌を遂げた歴史的背景が明らかとなってきている。しかし、SIVがヒトに感染し、病原性を示すようになった原因は、未だ解明がなされていない部分が多い。当該研究では、SIVが「種の壁」を乗り越えてヒトへ感染伝播する際に関わる自然免疫因子（群）およびHIV感染制御ヒト宿主因子（群）を同定することにより、新規エイズ治療法に向けての基盤確立に寄与すること、および今後の新興感染症に対するヒト宿主防御機構に対する理解を深めることが目的である。当該年度では、前年度に同定した2種の宿主因子の中でも、逆転写反応効率に影響をおよぼす因子について重点的に詳細な解析を行うと共に、本研究で得られた他のHIV感染制御候補因子群についても、順次機能評価を行った。具体的な進捗状況としては、（1）2種の宿主因子のうち、AMPKファミリーに属するAMPK-RPKの作用機序について詳細な解析を進めたところ、HIV感染過程において逆転写反応過程に影響をおよぼす原因として、AMPK-RPKが感染細胞内での逆転写反応の場であるHIVキャプシドコアの安定性に影響をおよぼすことが起因していることが明らかとなった。また、AMPK-RPKは、HIV感染後期過程において、ウイルスmRNA転写効率に影響をおよぼすことによって、ウイルスタンパク合成量を低下させることが明らかとなった。（2）もう一つの宿主因子として同定したアクチン結合タンパク質（ABP）について詳細な解析を進めたところ、HIV感染過程において、HIV吸着・侵入過程に影響をおよぼすHIV感染制御因子であることが明らかとなった。（3）当該研究で得られた宿主候補因子群の機能評価を順次行ったところ、新たに4種のHIV感染制御因子を得る事ができた。これらは、細胞膜に局在するものだけでなく、核近傍に局在するものも含まれていた。以上の成果より、当該研究事業3年目の目標としていた機能遺伝子発現抑制T細胞ライブラリーから得られたHIV感染制御因子であるAMPK-RPKおよびABPの詳細な機能解析とともに新たなHIV感染制御因子群を見出すことが出来た。

A. 研究目的

本研究では、HIV感染伝播に関わる自然免疫（群）およびSIVがヒトへ感染伝播する際に関わる自然免疫因子（群）を同定し、新規エイズ治療法に向けての基盤確立に寄与すること、および今後の新興感染症に対するヒト宿主防御機構の理解を深めることを目的とした。

現在までにヒトゲノム情報に立脚したHIV感染制御宿主因子探索法として、RNA干渉法によるgenome-wide screening法による研究成果が幾つか報告されているが、本来のHIV感染標的細胞を用いたものではなく、そのため自然感染におけるHIV感染伝播での役割については不明な点が多い。本研究では、HIV感染標的細胞であるTリンパ球を用いて機能遺伝子発現抑制Tリンパ球ライブラリーを構築し、これらライブラリーと正常Tリンパ球との間でのHIV

感染効率を比較検討し、HIV 感染制御抑制因子群と SIV 感染制御因子群とを同定することを試みる。また、SIV がヒト T 細胞における感染増殖能に影響を及ぼすウイルス側要因の同定も試みる。平成 22 年度は、RNA 干渉法（shRNA）を用いたヒトおよびサル機能遺伝子発現抑制 T 細胞ライブラリーの樹立に成功し、HIV および SIV 感染制御宿主因子候補群を多数得ることが出来た。平成 23 年度は、前年度に得られた HIV 感染制御宿主因子候補群の機能解析を進めた結果、2 種の機能遺伝子発現抑制 T 細胞における HIV 感染増殖効率の著しい低下が認められ、その中の 1 つは、逆転写反応過程に影響をおよぼし、もう 1 つは HIV 感染吸着・侵入過程に影響をおよぼす宿主因子であることを明らかにした。

平成 24 年度は、前年度に同定した 2 種の宿主因子の中でも、逆転写反応効率に影響をおよぼす因子について重点的に詳細な解析を行うと共に、本研究で得られた他の HIV 感染制御候補因子群についても、順次機能評価を行った。

B. 研究方法

（1）HIV-1 感染細胞内におけるウイルス DNA 合成量の解析：機能遺伝子発現抑制 T 細胞株に HIV-1 (NL4-3 株)を感染させ、24 時間後の感染細胞内で、逆転写反応を経て合成されたウイルス DNA 量について、*pol* および *env* の各領域におけるリアルタイム PCR 法にて測定した。

（2）Fate-of-capsid アッセイによる感染細胞内のウイルスコア安定性の解析:ヒト T 細胞株に HIV-1 を感染させ、8 時間後の感染細胞を Dounce Homogenizer にて細胞破砕後に細胞質分画を得た。この細胞質分画を、20%-60%シュクロースに重層した後、細胞質成分の比重分離を超遠心法にて行った。比重分離後、上部から 3 分画に分けて回収し、各分画に含まれているウイルスキャプシド（CA）タンパク量を Wetern blotting 法（WB 法）および ELISA 法を用いて検出した。

（3）HIV 感染細胞内におけるウイルス mRNA 合成量の解析：HEK293 細胞に pNL4-3 を導入することで、HIV 感染成立環境を擬似的に構築し、宿主転写因子を利用したウイルス mRNA 合成量を、i) unspliced form、ii)single-spliced form、iii) double-spliced form の 3 種各々に検出するリアルタイム PCR 法にて経時的に測定した。

（4）HIV 感染細胞内におけるウイルスタンパク量の解析：HEK293 細胞に pNL4-3 もしくは pNL4-3luc（ルシフェラーゼレポーターHIV）を導入し、ウイルスタンパク量の経時的変化を WB 法および化学発光検出法にて測定した。

（5）AMPK-RPK 再構築細胞における HIV 感染効率の解析：AMPK-RPK-KD 293 細胞に AMPK-RPK タンパク発現プラスミドを導入することで AMPK-RPK 再構築を行い、VSV-G/NL4-3luc シュードタイプ HIV-1 を感染させる。感染 24 時間後に感染細胞内のルシフェラーゼ活性を測定することで感染効率を検討した。

（倫理面への配慮）

本研究における遺伝子組み換え生物等を用いる実験については、必要に応じた東京医科歯科大学の機関承認および文部科学大臣承認を既に取得済みである。

C. 研究結果

前年度に同定した 2 種の HIV 感染制御因子のうち、AMP-activated protein kinase（AMPK）ファミリーに属する AMPK-related protein kinase（AMPK-RPK）について詳細な解析を進めた。前年度までに、(1) HIV 吸着・侵入過程には影響をおよぼしていない

こと、(2) AMPK-RPK 発現レベルと HIV 感染効率とに相関関係があること、(3) HIV 感染過程において、逆転写反応効率を示すウイルス cDNA 合成量を著しく低下させることを明らかにしてきた。

今年度は、第一に、逆転写反応過程に影響を与える作用機序について解析を進めた。現在までに、感染細胞内における逆転写反応が行われる場は、キャプシド (CA) コア内であることが分かっており、コアの安定性が逆転写反応効率と強く相関していることが明らかとなっている。そこで、AMPK-RPK が、逆転写反応が行われている際のコアの安定性に影響を及ぼしているかどうかを検討するため、従来の Fate-of-capsid assay 法を改良し、T 細胞内における CA コアの安定性について解析を行った (図1)。その結果、ヒト T 細胞内において、AMPK-RPK 発現レベルが低下した結果、CA コアの安定性が変化していることが明らかとなった (図1、分画#3、Parental と AMPK-RPK-KD との比較)。

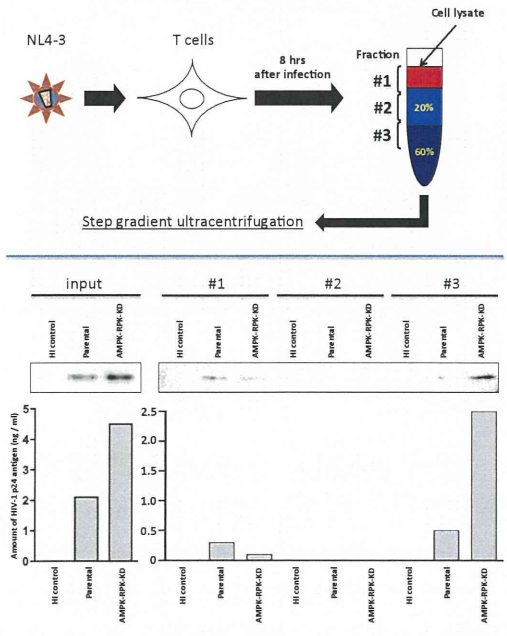

図 1. HIV 感染細胞内における CA コアの安定性に対する AMPK-RPK の影響. 上段 : MT-4/CCR5、Non-target shRNA、AMPK-RPK-KD 細胞に HIV-1(NL4-3 株)を感染させ、8 時間後に感染細胞の細胞質分画を抽出し、20-60%シュクロースの上に細胞質分画を重層し、超遠心法にて CA コアを分離した。CA コアは#3 分画に集積している。下段 : #1, #2, #3 の各分画について CA タンパクを検出する Western blotting を行った。下部のグラフは、各分画を ELISA 法にて定量を行った結果を示している。

第二に、AMPK-RPK の HIV 感染後期過程における影響を解析するために、HEK293 細胞に pNL4-3 DNA を導入した後に合成されるウイルスタンパク量に AMPK-RPK が影響をおよぼすか否かを検討した。その結果、培養上清中に産生される HIV CA p24 タンパク量だけでなく、AMPK-RPK の細胞内発現量が低下するに従い細胞内 HIV CA p24 タンパク量も低下することが明らかとなった。

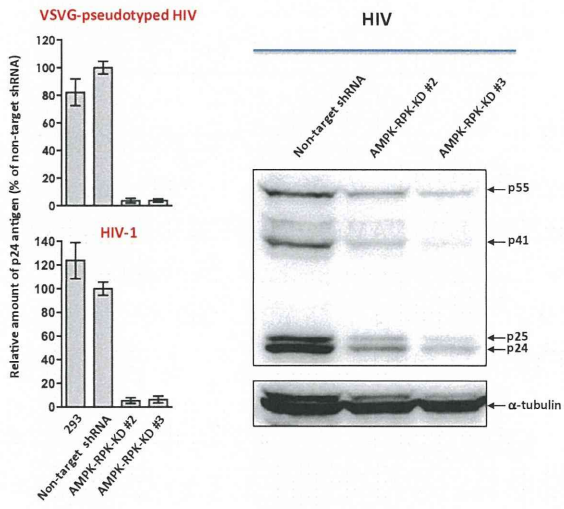

図 2. 細胞内 HIV gag タンパク発現量に対する AMPK-RPK の影響. 左側:293、non-target shRNA、AMPK-RPK-KD 細胞に、pNL4-3 をトランスフェクションし、24 時間後の培養上清中に含まれる HIV p24 タンパク量を ELISA 法にて定量した。右側 : 24 時間後の細胞内 HIV p24 タンパク量を WB 法にて検出した。

次に、AMPK-RPK がタンパク合成効

率に影響を及ぼしているか否かを検討するため、non-target shRNA コントロール細胞とAMPK-RPK細胞とを用いてウイルスタンパク合成量を経時的に比較検討したところ、ウイルスタンパク合成効率に影響を及ぼしていることが明らかとなった（図３）。

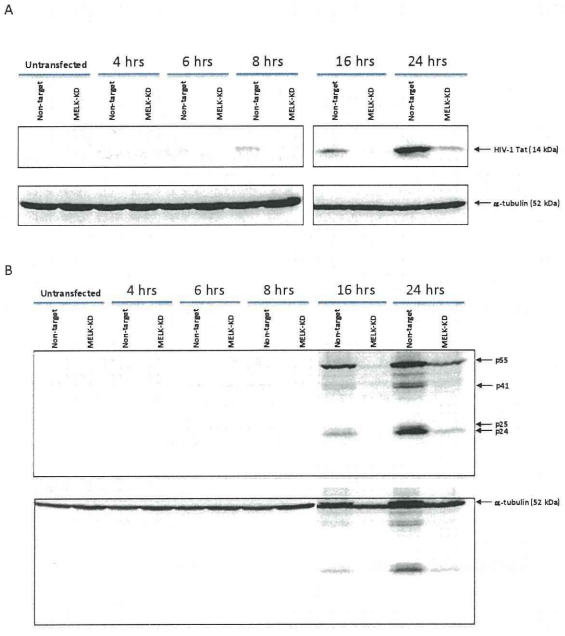

図３． HIV タンパク合成効率に対するAMPK-RPK の影響． 293 non-target shRNA およびAMPK-RPK-KD 細胞に、pNL4-3 をトランスフェクションし、24 時間後の細胞内 HIV Tat（A）および p24 （B）タンパク量をWB 法にて検出した。

AMPK-RPK が、ウイルスタンパク合成効率に影響を及ぼしている可能性が示唆されたことから、次にウイルス mRNA 合成効率に影響を及ぼしているか否かを検討した。具体的には、AMPK-RPK-KD 細胞内において、HIV-1 cDNA が宿主転写因子によって合成された mRNA に対して特異的かつ定量的に検出するリアルタイム PCR 法を用いて HIV-1 転写過程への影響を検討した。その結果、AMPK-RPK-KD 細胞内では、HIV-1 mRNA 合成効率が、non-target shRNA control 細胞と比較して顕著に低下することが明らかとなった（図４）。

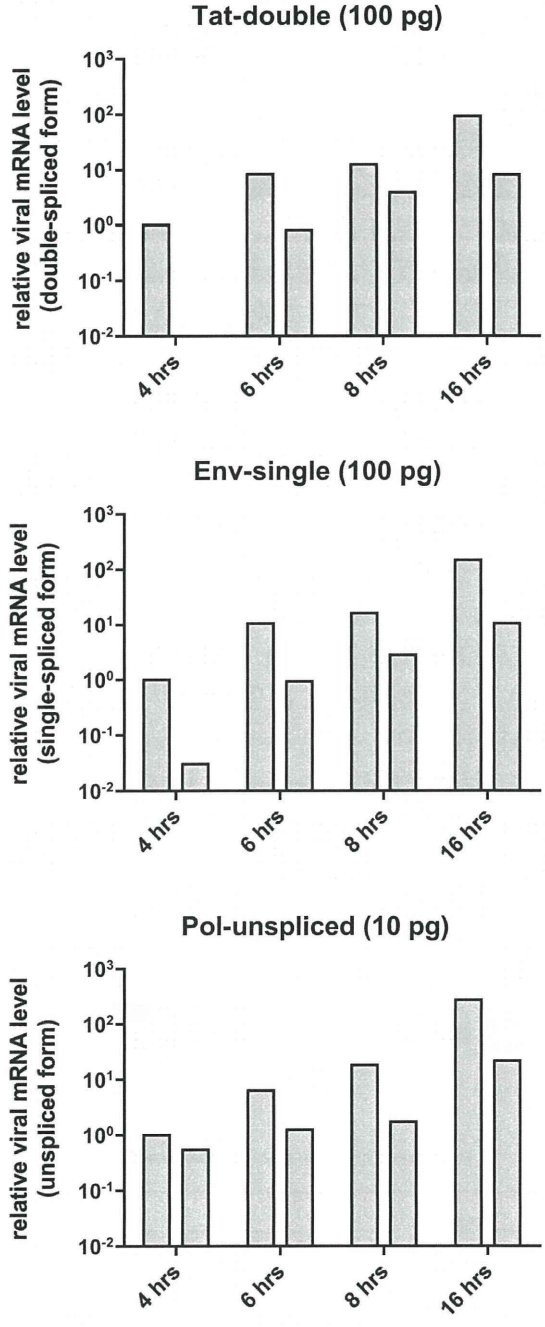

図４．HIV mRNA 合成効率に対するAMPK-RPK の影響．上段：293 non-target shRNA control 細胞（各時間における左側棒グラフ）と 293 AMPK-RPK-KD 細胞（各時間における右側棒グラフ）に pNL4-3 をトランスフェクションし、4、6、8、24 時間後の各時間において Total RNA を抽出し、リアルタイム RT-PCR 法を用いてウイルスmRNA合成量を定

量した。ウイルス mRNA 検出法としては、double-spliced form（上段）、single-spliced form（中段）、unspliced form（下段）の各 mRNA 形状を各々特異的に検出する方法を用いた。

本研究では、shRNA を用いた遺伝子発現制御法にて、標的遺伝子の機能解析を行っているため、shRNA が標的特異的に作用しているかどうかを確認する必要がある。そこで、AMPK-RPK が HIV 感染制御因子であることを更に検証するため、AMPK-RPK-KD 細胞内に AMPK-RPK タンパク発現プラスミドを導入することでAMPK-RPKタンパク再構築を行い、HIV 感染効率が復帰するかどうかを検討した。その結果、再構築することで、HIV 感染効率が有意に上昇することが明らかとなった（図５）。

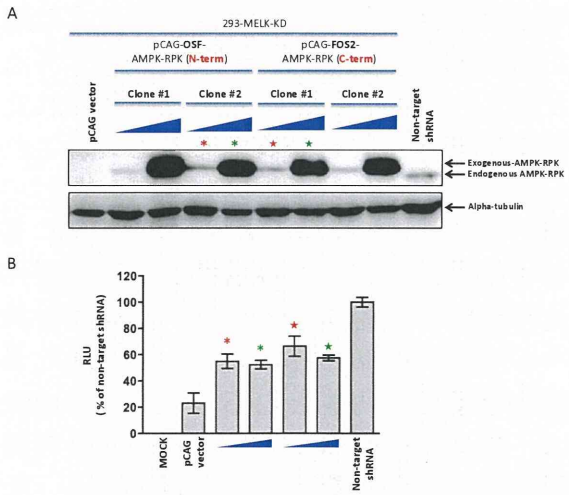

図５．AMPK-RPK-KD 細胞における HIV 感染効率に対する AMPK-RPK タンパク再構築の影響．293 AMPK-RPK-KD 細胞に AMPK-RPK タンパク発現プラスミド（pCAG-OSF/FOS2-AMPK-RPK）を遺伝子導入し（A）、導入２４時間後に VSV-G/NL43-luc を感染させ、感染細胞内のルシフェラーゼ活性を測定した（B）。

また、当該研究で AMPK-RPK と共に HIV 感染制御候補因子として見出されたアクチン結合タンパク（ABP）について、ABP が HIV 感染効率におよぼす影響についても検討した。具体的には、T リンパ球を用いて ABP 発現抑制細胞（ABP-KD）を樹立し、HIV 特異的および HIV 非特異的吸着・侵入過程におよぼす影響を検討した。その結果、HIV 特異的吸着・侵入過程に影響をおよぼす可能性が示唆された（図６、下段：HIV-1 envelope と VSV-G envelope との比較）。

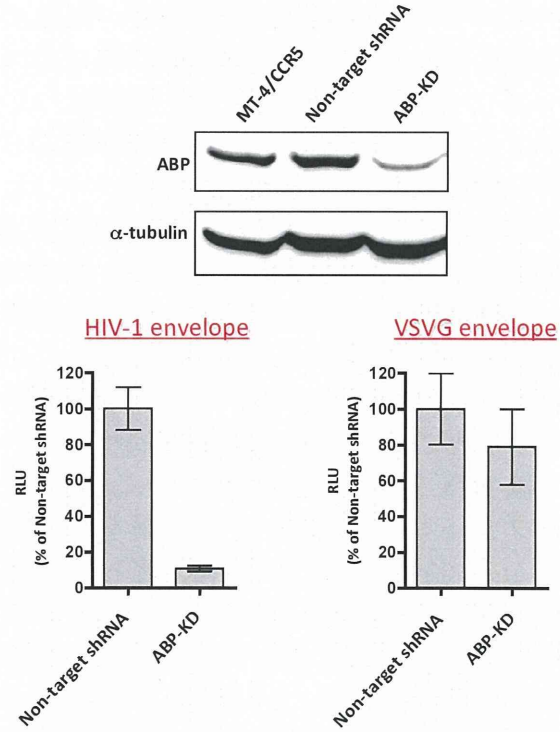

図６．HIV 感染効率に対する ABP の影響．ABP-KD T リンパ球（上段）に HIV-1 または VSV-G エンベロープを用いたシュードタイプ HIV を感染させた。感染２４時間後の感染細胞内のルシフェラーゼ活性を測定する事で感染効率を検討した（下段）。

以上の結果より、細胞内 AMPK-RPK 発現量が低下することで、HIV-1 感染細胞内における CA コアの安定性に影響を与えることで逆転写反応効率を低下させるだけでなく、HIV mRNA 合成効率にも影響をおよぼすことが明らかとなった。また、ABP は HIV-1 envelope 特異的なウイルス感染効率に影響するこ

とが明らかとなった。よって、AMPK-RPK は複数の HIV 感染過程に影響をおよぼす HIV-1 感染必須因子であり、ABP は HIV 感染における吸着・侵入過程に影響をおよぼす HIV 感染必須因子であることが示唆された。

D. 考察

現在までに、ヒトゲノム情報に立脚したエイズウイルス制御宿主因子探索は既に幾つかの成果が報告されているが、エイズウイルス標的細胞を用いたものではなく、そのため自然感染におけるエイズウイルス感染伝播での役割については不明な点が多い。当該研究事業の３年計画の３年目の研究成果については、２年目に得られた HIV-1 感染制御宿主因子候補群の機能解析を進めた。具体的な進捗状況としては、（１） 顕著な HIV 感染制御効果を示した因子群の１つである AMPK-RPK について詳細な解析を進めたところ、HIV 感染前期過程においては HIV 逆転写反応の場である HIV キャプシドコアの安定性に影響をおよぼすことで逆転写反応効率を著しく低下し、HIV 感染後期過程においては HIV 転写効率に影響をおよぼすことで、HIV 産生効率が低下していることが明らかとなった。この HIV mRNA 合成効率への影響については、HIV 特異的スプライシング過程や HIV Tat タンパクによる転写活性化メカニズム、更には、HIV mRNA 合成に必須な転写因子活性に影響をおよぼしていないことが分かってきたが、現段階ではその作用機序解明までには至っていないが、これらの研究結果は、１つの細胞内因子が、異なる作用機序でもって HIV 感染過程に影響をおよぼしていることを示している。また、AMPK-RPK の HIV 感染制御効果は、AMPK ファミリーの酵素活性阻害剤の影響を受けないことから、AMPK-RPK 特異的な作用であると考えられる。

（２）当該研究にて、AMPK-RPK と共に T 細胞株において感染効率の顕著な低下が認められた ABP の作用機序について解析を進めたところ、HIV-1 感染過程特異的な細胞侵入時に影響をおよぼすことが明らかとなった。（３）その他の HIV-1 感染増殖効率低下が認められた T 細胞群については、その中に混在している shRNA の単独発現 T 細胞株を順次樹立して解析を進めているところである。これらの結果は、当初の研究計画の３年目に予定していた研究進捗度と同程度の進捗状況であり、また新たな研究成果が得られたことから、当該研究事業３年目の目標をほぼ予定通り達成出来たと考えられる。

近年、インフルエンザの異種間感染や SARS による人的被害の状況を踏まえ、新興・再興および人獣共通感染症に対する適切な予防策を一刻も早く講じる必要性が急務となっており、このことは厚生労働行政の最重要課題の一つであると考えられる。当該研究から同定された宿主制御因子群を公表することで、これらを「バイオロジカルプローブ」として用いた新たなエイズ治療法の確立に寄与する事が出来、この治療法を薬剤併用化学療法と併用することで、薬剤耐性株への効果的な治療法の確立に向けた具体的な議論が可能となる。これらの成果は、様々な病原体に対する種間感染の発展的な解析が可能になるだけでなく、それらの新規予防・治療法の開発に大きく寄与することが予想される。

以上の事から、当該研究を３年間遂行することで、エイズウイルス感染制御宿主因子を利用した新たなエイズ治療法の基盤確立に向けた情報を集積することが出来ると考えられる。

E. 結論

ゲノムワイドスクリーニング法による HIV 感染制御因子群の探索を目的とした本研究において、新たな HIV 感染

制御因子として、AMPK-RPK および ABP を同定した。AMPK-RPK は、複数の HIV 感染過程に影響をおよぼすことで、HIV 感染増殖伝播効率に影響をおよぼし、ABP は HIV 特異的吸着・侵入過程に影響をおよぼす HIV 感染必須因子であるという結果を得た。これらの特異的機能阻害剤を開発することによって、新規エイズ治療法の開発に結びつく重要な成果であると考えられる。

F. 健康危険情報

該当なし

G. 研究発表

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2. 学会発表

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H. 知的財産権の出願・登録状況

該当なし

別紙４

研究成果の刊行に関する一覧表レイアウト

書籍

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SIV replication in human cells

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Current human immunodeficiency virus type 1 pandemic is believed to originate from cross-species transmission of simian immunodeficiency virus (SIV) into human population. Such cross-species transmission, however, is not efficient in general, because viral replication is modulated by host cell factors, with the species-specificity of these factors affecting viral tropism. An understanding of those host cell factors that affect viral replication contributes to elucidation of the mechanism for determination of viral tropism. This review will focus an anti-viral effect of ApoB mRNA editing catalytic subunit, tripartite motif protein 5 alpha, and cyclophilins on SIV replication and provide insight into the mechanism of species-specific barriers against viral infection in human cells. It will then present our current understanding of the mechanism that may explain zoonotic transmission of retroviruses.

Keywords: HIV-1, SIV, APOBEC3G, TRIM5α, cyclophilin A, cyclophilin B

INTRODUCTION

There is significant evidence that the ongoing worldwide acquired immunodeficiency syndrome (AIDS) epidemic was caused by cross-species transmission of simian immunodeficiency viruses (SIVs) into the human population. Replication of primate lentiviruses in their natural hosts is generally non-pathogenic; however, cross-species transmission of these viruses can result in highly pathogenic phenotypes. How and when this transmission occurred is still debated but it is now generally accepted that HIV-2 originated from a sooty mangabeys strain of SIV (SIVsm; Hirsch et al., 1989; Chen et al., 1996) while HIV-1 appears to have originated from a chimpanzee strain of SIV (SIVcpz; Gao et al., 1999). Zoonotic transmission of SIVs, however, is not common and is controlled by host factors that generally prohibit SIV replication in human hosts and many human-derived cell lines.

Viral replication is modulated by host cell factors, with the species-specificity of these factors affecting viral tropism. Some of these host factors can restrict viral replication and the anti-viral systems mediated by such host restriction factors, termed intrinsic immunity, play an important role in determining species-specific barriers against viral infection. For instance, Fv-1 in mice is known to restrict replication of a murine leukemia virus (Rein et al., 1976; Gautsch et al., 1978; Towers et al., 2000) and tripartite interaction motif 5α (TRIM5α) recently has been found to be responsible for restricting HIV-1 but not SIV infection in Old World monkey (OWM) cells (Hatziioannou et al., 2004b; Keckesova et al., 2004; Stremlau et al., 2004; Yap et al., 2004; Song et al., 2005; Ylinen et al., 2005). Restriction of retroviral replication by these host cell factors takes place after viral entry, but before the integration step, and the viral determinants for this type of restriction have been mapped to the capsid (CA) protein (Gautsch et al., 1978; Kozak and Chakraborti, 1996; Towers et al., 2000; Goff, 2004; Stremlau et al., 2006). Two recent studies showed that the cellular protein SAMHD1 is myeloid-lineage cell-specific HIV-1 restriction factor counteracted by Vpx proteins from HIV-2 and SIVsm (Hrecka et al., 2011; Laguette et al., 2011). Restriction of lentivirus infection by SAMHD1 is likely to take place at the reverse transcription step. Another anti-retroviral protein, tetherin (also referred to as BST-2, CD317, or HM1.24) inhibits retrovirus release and is antagonized by HIV-1 Vpu protein, Nef protein of many SIVs, or Env protein of HIV-2 (Neil et al., 2008; Le Tortorec and Neil, 2009; Zhang et al., 2009). Understanding how host cell factors affect viral replication, positively or negatively, would contribute to elucidating the molecular mechanism that determines viral tropism. Here, we discuss an anti-viral effect of ApoB mRNA editing catalytic subunit (APOBEC), TRIM5α, and cyclophilins (Cyps) on SIV replication.

APOBEC: ENZYMATIC RESTRICTION FACTOR THAT TARGET RETROVIRUSES

Replication of HIV-1 in primary CD4+ T cells, monocyte, and some immortalized T cell lines depends on the presence of the HIV-1 accessory gene product, Vif (standing for virus infectivity factor; Fisher et al., 1987; Strebel et al., 1987), and it works in a host cell-specific manner. Vif is required for enhanced HIV-1 replication in some cell types called non-permissive cells. In contrast, HIV-1 replication is Vif-independent in permissive cells (Akari et al., 1992; Fan and Peden, 1992; Gabuzda et al., 1992; Blanc et al., 1993; Sakai et al., 1993; von Schwedler et al., 1993; Borman et al., 1995). Recently, some cytidine deaminases were identified as a new class of host restriction factors that target retroviruses such as HIV-1 or SIV (Harris and Liddament, 2004; Cullen, 2006). APOBEC3G (Apo3G), a member of the APOBEC family of cytidine deaminases, is the first identified enzymatic restriction factor and the determinant that makes cells permissive or non-permissive. Apo3G is also a host factor that restricts replication of human and simian lentiviruses in their respective target cells. Unlike TRIM5α or Fv-1, Apo3G does not exert its anti-viral activity by targeting the viral CA protein, but it has to be incorporated

into a newly synthesized virion during a production step, and then inhibits virus replication by targeting single-stranded viral cDNA during a subsequent infection step. HIV-1 counteracts Apo3G with Vif expression. During the production of progeny virions, Vif binds to Apo3G and induces Apo3G's proteasomal degradation, resulting in the decreased steady-state levels of human Apo3G (hApo3G; Yu et al., 2003).

There are several anti-retroviral mechanisms of Apo3G against HIV-1 infection. First, Apo3G-containing virus can accumulate in a large number of substitutions that register as cytidine (C) to deoxyuridine (dU) in a virus minus-strand during reverse transcription, resulting guanine (G) to adenine (A) mutations in a viral plus-strand, known as "G-to-A hypermutation" (Harris et al., 2003; Lecossier et al., 2003; Mangeat et al., 2003; Mariani et al., 2003; Zhang et al., 2003; Yu et al., 2004b). Second, Apo3G can inhibit tRNA annealing or tRNA processing during reverse transcription (Guo et al., 2006, 2007; Mbisa et al., 2007). Third, Apo3G inhibits DNA strand transfer or integration (Li et al., 2007; Luo et al., 2007; Mbisa et al., 2007). Although Apo3G has the most potent anti-HIV-1 activity among the APOBEC family of proteins, another member of the family, APOBEC3F (Apo3F) was shown to inhibit HIV-1 infection in the absence of Vif (Bishop et al., 2004a; Liddament et al., 2004; Wiegand et al., 2004; Zheng et al., 2004), whereas APOBEC3B (Apo3B) can inhibit HIV-1 infection in both the presence and absence of Vif (Bishop et al., 2004a; Doehle et al., 2005; Rose et al., 2005).

Although we can imagine the broad range of anti-retroviral activity of APOBEC family because APOBEC proteins from non-human species can also inhibit HIV-1 infection (Mariani et al., 2003; Bishop et al., 2004a,b; Wiegand et al., 2004; Cullen, 2006), the Vif-Apo3G interaction is thought to be species-specific (Simon et al., 1998; Mariani et al., 2003). Accordingly, hApo3G is insensitive to SIVagm Vif while African green monkey Apo3G (agmApo3G) is insensitive to HIV-1 Vif and the determinant of this species-specificity depends on amino acid 128 of hApo3G and agmApo3G (Mariani et al., 2003; Bogerd et al., 2004; Mangeat et al., 2004; Schrofelbauer et al., 2004; Xu et al., 2004).

However, such species-specificity is not strictly controlled, for example, a report from the laboratory of Klaus Strebel demonstrated that SIVagm Vif supported replication of SIVagm virus in the hApo3G-positive human A3.01 T cell line (Takeuchi et al., 2005). Replication of *vif*-defective SIVagm in A3.01 cells was severely restricted, resulted in an accumulation of cytidine deaminase-induced G-to-A mutations in SIVagm genome (Takeuchi et al., 2005).

Moreover, two independent groups showed that the different APOBEC3 family members function to neutralize specific lentiviruses (Yu et al., 2004a; Dang et al., 2006). One report from the lab of Dr. Nathaniel R. Landau showed that APOBEC3B and APOBEC3C were potent inhibitors of SIV (Yu et al., 2004a). Both enzymes were efficiently encapsidated by HIV-1 and SIV. Another report from the lab of Dr. Yong-Hui Zheng demonstrated that APOBEC3DE blocked the replication of both HIV-1 and SIV but not that of MLV (Dang et al., 2006) and APOBEC3H inhibited the replication of HIV-1 by a cytidine deamination-independent mechanism (Dang et al., 2008). These findings raise the possibility that the various APOBEC3 family members protect against different lentiviruses and point to a possible role in the zoonotic transmission of SIV.

TRIM5α: FV-1-TYPE HOST FACTOR RESTRICTING HIV-1 IN PRIMATE CELLS

The host protein which dictates Ref1 activity was identified as an α-isoform of rhesus macaque TRIM5 protein by the laboratory of Dr. Joseph Sodroski (Stremlau et al., 2004). TRIM5 is a member of the TRIM family of proteins, and has RING, B-box 2, and coiled-coil as common and conserved domains among the family and B30.2 (PRYSPRY) domain on its C-terminal region (Nisole et al., 2005). Subsequently, the human and non-human primate homologs of TRIM5α were shown to restrict retroviruses, such as N-MLV, and equine infectious anemia virus (Hatziioannou et al., 2004b; Keckesova et al., 2004; Perron et al., 2004; Yap et al., 2004; Song et al., 2005; Ylinen et al., 2005; Si et al., 2006). Rhesus monkey TRIM5α (rhTRIM5α) has strong anti-HIV-1 activity but only modestly restricts SIV isolated from a macaque monkey (SIVmac) and does not block MLV infection, whereas its human homolog does not restrict HIV-1 infection.

TRIM5α recognizes incoming viral cores, but not a monomeric CA protein, thorough its B30.2 (PRYSPRY) domain. B-box 2 and coiled-coil domains are required for TRIM5α multimerization, and both coiled-coil and B30.2 (PRYSPRY) domains are essential for viral core binding (Reymond et al., 2001; Stremlau et al., 2006). TRIM5α captures HIV-1 core at a very early step(s) after infection, immediately after the release of the core into cytoplasm. To restrict HIV-1 infection and to recognize viral core, TRIM5α must oligomerize through its B-box 2 and coiled-coil domains (Mische et al., 2005; Li and Sodroski, 2008). Its RING domain has E3 ubiquitin ligase activity. It self-ubiquitination occurs TRIM5α is quickly degraded (Diaz-Griffero et al., 2006). This rapid degradation of TRIM5α is not required for post-entry restriction since replacement of TRIM5α RING domain with the corresponding domain of TRIM21, which has lower self-ubiquitination activity and a longer half-life than TRIM5α did not alter the anti-viral activity (Kar et al., 2008). Recently, the laboratory of Dr. Mark Yeager discussed a novel architecture made with dimers of TRIM5-21R. TRIM5α-21R forms a dimer through its B-box 2 and coiled-coil domains, and these dimers form six-sided rings on CA lattices to promote rapid core disassembly (Ganser-Pornillos et al., 2011). Overexpression of TRIM5α leads to the formation of cytoplasmic bodies and is believed to be required for its anti-viral activity (Stremlau et al., 2006; Campbell et al., 2008). During TRIM5α-mediated post-entry restriction, disassembly of viral cores is induced too quickly and the accumulation of viral RT-products is reduced (Stremlau et al., 2006). On the other hand, MG132 treatment inhibited quick-disassembly, yet HIV-1 infectivity was still restricted. Two reports showed that TRIM5α could block not only viral cDNA accumulation but also the nuclear import of viral cDNA (Berthoux et al., 2004; Wu et al., 2006). Thus, TRIM5α-mediated post-entry restriction is thought to have at least two phases: (i) TRIM5α induces rapid disassembly of viral core in a proteasome-dependent manner and (ii) TRIM5α degrades HIV-1 cDNAs in a proteasome-independent manner. The determinant of specificity and magnitude of the post-entry

restriction lies on B30.2 (PRYSPRY) domain. Previous report showed that TRIM5α alleles did not cluster by species between rhesus macaques and sooty mangabeys and none of the alleles from either species restricted SIV, suggesting that there is little effect of rhTRIM5α on transmission of SIVsm within species (Newman et al., 2006). Recently, Pacheco et al. (2010) reported that New World monkey (NWM) TRIM5α restricts foamy virus infection. Another consideration is the clinical significance of TRIM5α against AIDS in human. Moreover, several reports showed that the efficacy of TRIM5α-mediated suppression of HIV-1 replication might interfere with disease progression of AIDS in humans (van Manen et al., 2008; Cagliani et al., 2010; Takeuchi et al., 2012). Thus, TRIM5α-mediated restriction may be a multi-step process in retrovirus replication with the relationship between other host factor(s).

Recently, the lab of Dr. Yasuhiro Ikeda reported that rhesus macaque TRIM5α also inhibits HIV-1 production by inducing the degradation of a viral precursor Gag protein (Sakuma et al., 2007). To restrict HIV-1 production, amino acid residues in B-box 2 and coiled-coil domains dictated the specificity of the restriction. In the late restriction, the accumulation of HIV-1 RNA was not affected but the accumulation of precursor Gag was inhibited in an ubiquitin–proteasome-independent manner. This TRIM5α-mediated late-restriction is still controversial (Zhang et al., 2008), yet it is conceivable that TRIM5α restricts HIV-1 infection and production in two distinct mechanisms. Although TRIM5α restricts HIV-1 infection in a broad range of cells, its late restriction involved transient overexpression (Sakuma et al., 2007).

Here is another notable class of the TRIM family called TRIM-Cyp isolated from NWM. A report from the laboratory of Dr. Jeremy Luban demonstrated that owl monkey cells express TRIM-Cyp that restricts HIV-1 infection (Sayah et al., 2004). Although TRIM-Cyp has a cyclophilin A (CypA) sequence in its C-terminal region instead of B30.2 (PRYSPRY) domain that dictates the specificity and the magnitude of post-entry restriction in OWM TRIM5α-mediated post-entry restriction, it recognizes incoming core structure and restricts HIV-1 infection (Stremlau et al., 2006). Recently, TRIM-Cyp mRNA was also detected in a rhesus macaque cell, and overexpressed rhesus TRIM-Cyp restricts HIV-1 infection and production (Newman et al., 2006; Brennan et al., 2008; Wilson et al., 2008; Dietrich et al., 2010).

Unlike other restriction factors, there is no known accessory gene product of HIV-1 to antagonize TRIM5α-mediated restrictions. Indeed, human TRIM5α has only a modest restriction activity against HIV-1 infection. TRIM5 proteins from several NWM species restrict infection by SIVmac and SIVagm (Song et al., 2005). This suggests that TRIM5α could be a key molecule of the species-species barrier.

CYCLOPHILINS: HOST FACTORS INVOLVED IN RETROVIRUS REPLICATION

Cyclophilins are ubiquitous proteins and first identified as the target of cyclosporine A (CsA), an immunosuppressive reagent (Takahashi et al., 1989). CypA has proline-isomerase activity that catalyzes the *cis–trans* isomerization of proline residue (Fischer et al., 1989). The binding of CsA to CypA inhibits this isomerase activity (Takahashi et al., 1989). In retrovirus replication, CypA was found to bind HIV-1 CA in the yeast two-hybrid system (Luban et al., 1993). The sequence Ala88-Gly89-Pro90-Ile91 of CA protein is the major fragment bound to the active site of CypA (Franke et al., 1994; Gamble et al., 1996; Zhao et al., 1997). Interestingly, The peptidyl-prolyl bond between Gly89 and Pro90 of the CA fragment has a *trans* conformation, in contrast to the *cis* conformation observed in other known CypA–peptide complexes (Zhao et al., 1997; Bosco et al., 2002), and Gly89 preceding Pro90 has an unfavorable backbone formation usually only adopted by glycine, suggesting that special Gly89-Pro90 sequence but not other Gly-Pro motif is required for the binding of CA protein to CypA. Therefore, CypA might be likely to act as a molecular chaperone but not a *cis–trans* isomerase (Zhao et al., 1997). However, one report showed that CypA does not only bind CA protein but also catalyzes efficiently *cis–trans* isomerization of Gly89-Pro90 peptidyl-prolyl bond (Bosco et al., 2002). The relationship between the Gly89-Pro90 bond and catalysis of *cis–trans* isomerization by CypA remains unclear.

It has been well established that CypA promotes an early step of HIV-1 infection in human cells (Franke et al., 1994; Thali et al., 1994; Braaten et al., 1996a,c; Franke and Luban, 1996; Braaten and Luban, 2001; Sokolskaja et al., 2004; Hatziioannou et al., 2005). CypA is efficiently encapsidated into HIV-1 produced from infected cells through interaction with the CA domains of the Gag polyprotein and disruption of CypA incorporation into virions by CsA or HIV-1 Gag mutants caused a decrease in replication efficiency (Franke et al., 1994; Thali et al., 1994; Ott et al., 1995; Braaten et al., 1996a; Bukovsky et al., 1997; Ackerson et al., 1998; Braaten and Luban, 2001). It is still unclear how CypA is efficiently packaged into HIV-1 virion, but several reports showed that both dimerization of CA and multimerization of CypA are required for efficient interaction (Colgan et al., 1996; Javanbakht et al., 2007). Although CA-CypA interaction is required for infectivity, the important point is that CypA interacts with incoming HIV-1 cores in newly infected target cells rather than during HIV-1 budding from the virion producer cells, indicating that target cell CypA promotes HIV-1 infectivity (Kootstra et al., 2003; Towers et al., 2003; Sokolskaja et al., 2004).

CypA-dependent virus replication is only limited to retroviruses which encode CA that binds CypA. In fact, only those retroviruses are dependent upon CypA for replication (Luban et al., 1993; Franke et al., 1994; Thali et al., 1994; Braaten et al., 1996c; Franke and Luban, 1996). These observations suggested that CA–CypA interaction might contribute tropism determinants for retroviruses. HIV-1 infection in non-human primate cells is blocked prior to reverse transcription after virus entry (Shibata et al., 1995; Himathongkham and Luciw, 1996; Hofmann et al., 1999; Besnier et al., 2002; Cowan et al., 2002; Munk et al., 2002; Hatziioannou et al., 2003; Towers et al., 2003). This restriction is thought to be the same step in the retrovirus life cycle where CypA works (Braaten et al., 1996b). Indeed, analysis of CypA-binding region of CA with chimeric viruses of HIV-1 and SIV showed the viral determinant for species-specificity (Shibata et al., 1991, 1995; Dorfman and Gottlinger, 1996; Bukovsky et al., 1997;

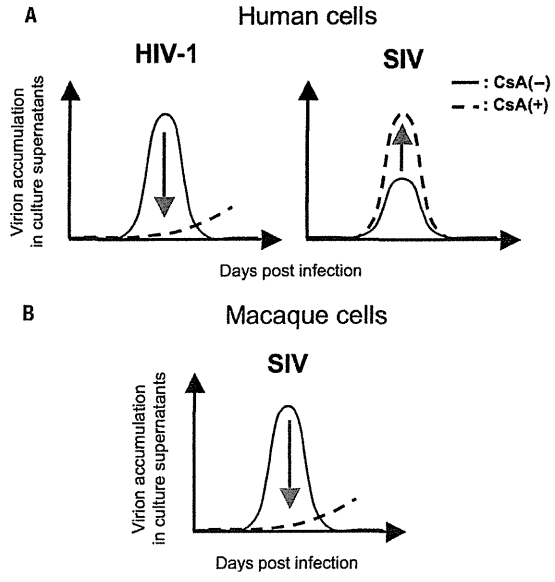

FIGURE 1 | A schema for the effect of CsA on HIV/SIV replication in human/macaque cells. (A) CsA treatment impairs the replication of HIV-1 (left panel) but enhances SIV replication (right panel) in human cells. **(B)** CsA treatment inhibits SIV replication in macaque cells. The solid line indicates virion accumulation of culture supernatant in the absence of CsA and the broken line indicates that of culture supernatant in the presence of CsA.

Cowan et al., 2002; Kootstra et al., 2003; Owens et al., 2003, 2004; Towers et al., 2003; Berthoux et al., 2004; Hatziioannou et al., 2004a, 2006; Ikeda et al., 2004; Sayah et al., 2004; Stremlau et al., 2004; Kamada et al., 2006).

Human CypA is required for efficient HIV-1 infection but not SIV. There is no known role for CypA in SIV infection in human cells. Recently, the first report from the laboratory of Klaus Strebel showed that human CypA acts as restriction factor against the infection of two SIVs (SIVmac and SIVagm) in human cells, and Vif protein of two SIVs counteracts a CypA-imposed inhibition against the infection of two SIV strains with exclusion of CypA from SIV virion (Takeuchi et al., 2007). This phenomenon is different from the function of SIVagm Vif against hApo3G previously reported from the same laboratory (Takeuchi et al., 2005) because they used human cells lacking detectable deaminase activity.

Moreover, a recent report showed a species-specific effect of CsA, a peptidyl-prolyl *cis–trans* isomerase (PPIase) inhibitor, on SIV replication, implying a possible contribution of Cyps to the determination of SIV tropism (**Figure 1**; Takeuchi et al., 2012). They demonstrated a host species-specific effect of CypA on SIV replication: CypA affects the replication of two SIVs (SIVmac and SIVagm) negatively in human cells but positively in macaque cells (**Figure 1**). Further analysis indicated that the infection of two SIVs was not significantly affected by CypA but inhibited by cyclophilin B (CypB), another PPIase, in human cells (**Figure 2A**; Takeuchi et al., 2012). In contrast, CypA is likely to have positive

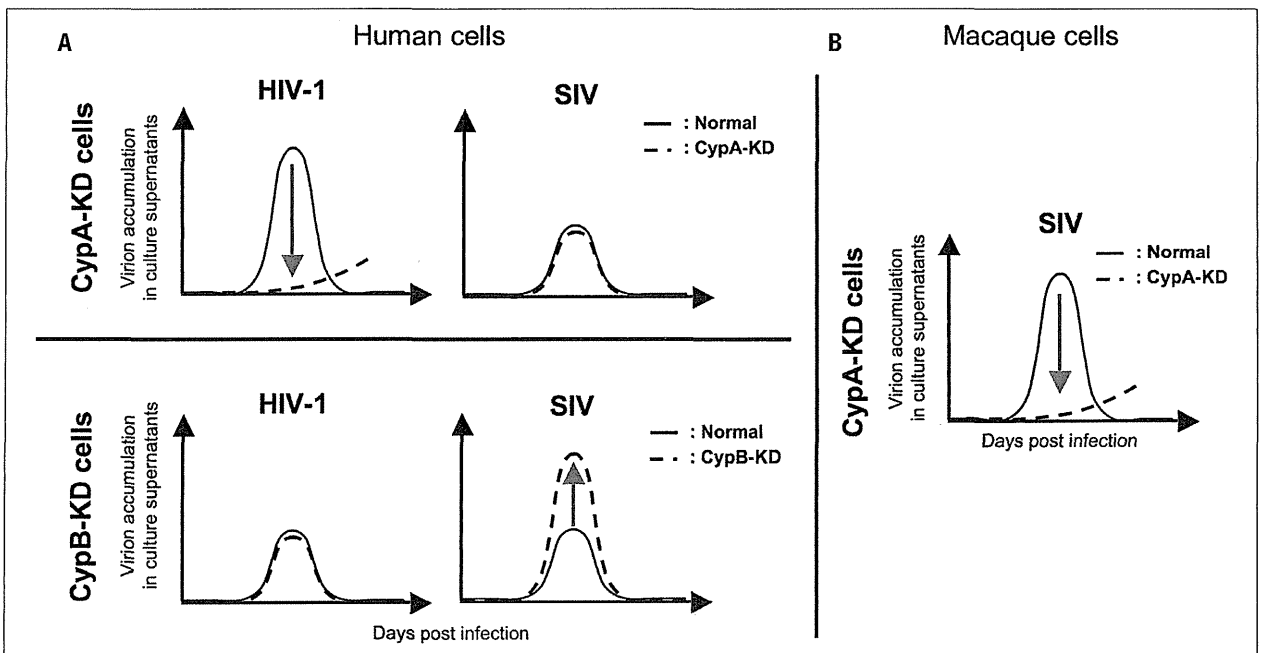

FIGURE 2 | A schema for the effect of cyclophilin A and cyclophilin B on HIV/SIV replication in human/macaque cells. (A) CypA knock-down (CypA-KD) impairs the replication of HIV-1 (upper left panel). In contrast, SIV replication is not reduced but rather enhanced by CypA knock-down (upper right panel). CypB knock-down (CypB-KD) shows no significant effect on HIV-1 replication (lower left panel) but enhances the replication of SIV (lower right panel). **(B)** CypA-KD inhibits SIV infection. The solid line indicates virion accumulation of culture supernatant produced from normal cells and the broken line indicates that of culture supernatant produced from CypA or CypB knock-down cells.

effects on the infection of two SIVs in macaque cells (**Figure 2B**; Takeuchi et al., 2012). These results suggest that Cyps might have a host species-specific effect of Cyps on SIV replication and provide insight into the mechanism of species-specific barriers against viral infection.

CONCLUDING REMARKS

Viral replication is modulated by host cell factors. Many of these factors function in a species-specific manner. On the other hand, there exist host factors that restrict viral replication. The antiviral system mediated by some of these restriction factors, termed intrinsic immunity, which is distinguished from the conventional innate and adaptive immunity has been indicated to play an important role in making species-specific barriers against viral infection. As discussed in this review, we describe the current progress in understanding of such restriction factors against retroviral replication, especially focusing on TRIM5α and APOBEC whose anti-retroviral effects have recently been recognized. Additionally, we mentioned a host species-specific effect of Cyps including CypA and CypB on SIV replication. Such restriction factors would play an important role in determining species-specific barriers against viral infection.

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