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２３年度総括報告書

研究代表者　武内　寛明
東京医科歯科大学　医歯学総合研究科　ウイルス制御学分野

平成２４（２０１２）年５月

目次

厚生労働科学研究費補助金（エイズ対策研究事業）
総括研究報告書

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

研究代表者　武内　寛明
東京医科歯科大学　助教

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）HIV-1感染制御因子候補群から単一機能遺伝子発現抑制T細胞株を計6株樹立した。（2）（1）で樹立した機能遺伝子発現抑制T細胞株を用いてHIV-1感染実験を行った結果、6種類のうち2種類のT細胞株において感染効率の顕著な低下が認められた。そこで、この2種の宿主因子の作用機序について解析を進めたところ、1つはHIV-1の細胞侵入時に影響をおよぼし、もう1つはHIV-1の細胞侵入後の逆転写反応に影響をおよぼすことが明らかとなった。また、それ以外の4種についても、感染効率が50%以上低下することが明らかとなった。これらの結果は、本研究で推し進めている、機能遺伝子発現抑制T細胞ライブラリーを用いたHIV-1感染制御因子（群）の探索法の妥当性を示していると考えられる。以上の成果より、当該研究事業2年目の目標としていたT細胞ライブラリーから得られたHIV感染制御因子候補群からHIV-1感染制御因子群の同定およびそれらの詳細な機能解析を進めることが出来たと考えられる。

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。

B. 研究方法

（１）宿主機能遺伝子発現抑制T細胞ライブラリーの樹立およびHIV/SIV感染制御宿主因子群の探索：宿主遺伝子（約１万５千遺伝子）を標的とした short hairpin RNA (shRNA)ライブラリーを発現するカセットをT細胞ゲノムへ組み込むことで、shRNA ライブラリー安定発現T細胞株を樹立した。次に、これらT細胞ライブラリーを用いて、HIVおよびSIV感染耐性T細胞群（semi-clonal population）を選択した。その後、各々の細胞群が保持しているshRNA配列(群)を解析し、shRNA 配列が標的としている宿主機能遺伝子を同定した。

（２）単独shRNA発現T細胞株の樹立：HIV 感染増殖効率が著しく低下している細胞群（semi-clonal population）の中で、6種のshRNAが混在している細胞群について、各々のshRNA単独発現T細胞株を樹立した。

（３）HIV-1感染効率の解析：（２）で樹立した各T細胞株に、$HIV\text{-}1_{IIIB}$ env-pseudotyped HIV-1 (IIIBenv/luciferase-reporter HIV-1) またはVSVG-pseudotyped HIV-1 (VSVG/luciferase-reporter HIV-1)を感染させ、24時間後のluciferase活性を測定した。

（４）HIV-1増殖能の解析：各T細胞株に、HIV-1を感染させた。その後継時的に培養上清を回収し、それらに含まれるウイルス由来の逆転写酵素（RT）活性を測定した。

（５）HIV-1感染細胞内におけるウイルスDNA合成量の解析：（２）で樹立した各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。

（６）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CAタンパク量を Wetern blotting 法およびELISA法を用いて検出した。

（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

HIV感染耐性T細胞ライブラリーを希釈して再培養を行った後に得られた細胞集団（semi-clonal cell population）のウイルス既感染の有無、およびshRNAのoff-target効果によるウイルス感染必須レセプターであるCD4/CXCR4/CCR5の細胞膜表面発現レベルへの影響について、正常MT-4/CCR5細胞とともに比較検討を行った結果、HIV感染耐性細胞はHIV非感染細胞であり、かつHIV感染標的細胞としての性状を維持していることが判明した。これら各細胞集団に含まれるshRNAの配列は、4〜8種類含まれており、複数のshRNA発現細胞が混在していると考えられる。そこで、HIV感染増殖効率が著しく低下している細胞群（図１）の中で、6種のshRNAが混在している細胞群について、各々のshRNA単独発現T細胞株を樹立した。

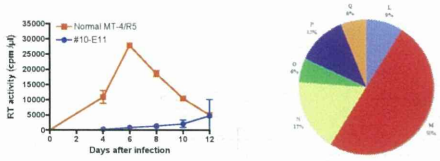

図 1. HIV 感染増殖効率に対する感染制御機能遺伝子群の影響. 6 種の機能遺伝子を標的としたT細胞ライブラリー集団(#10-E11)および正常 T 細胞 (Normal MT-4/R5) に NL4-3 を感染させ、経時的に培養上清中の RT activity を測定した。

次に、shRNA 単独発現 T 細胞株を用いて HIV-1 感染実験 (single-round infection) を行った結果、6種類のうち2種類の shRNA 単独発現 T 細胞株において感染効率の顕著な低下が認められた。また、それ以外の4種についても、感染効率が 50%以上低下することが明らかとなった。そこで、感染効率が顕著に低下した2種のうち、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。

AMPK-RPK がHIV-1感染制御因子となり得るかどうかを解析するために、内在性 AMPK-RPK の発現抑制 T 細胞株 (AMPK-RPK-KD) を樹立し、HIV-1 感染実験(single-round infection)を行った。まず、X4-tropic HIV-1 の感染経路である CD4/CXCR4 分子を利用した細胞侵入経路および VSVG 分子を利用した別の感染経路を利用して細胞侵入後の HIV-1 生活環に影響を及ぼすか否かを検討したところ、いずれの HIV-1 細胞内侵入経路においても HIV-1 生活環に影響をおよぼすことが分かった (図2)。

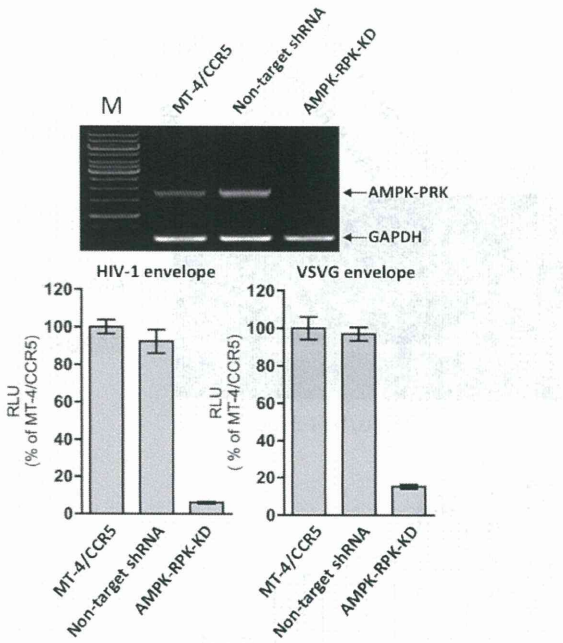

図2. HIV 感染効率に対する AMPK-RPK の影響. 上段:AMPK-RPK を標的とした shRNA を用いて AMPK-RPK-KD 細胞を作製し、MT-4/CCR5 細胞および shRNA コントロール細胞 (Non-target shRNA) の内在性 AMPK-RPK の mRNA 量と比較した結果。下段:MT-4/CCR5、Non-target shRNA、AMPK-RPK-KD 細胞に IIIB/NL-luc および VSVG/NL-luc HIV-1 を感染させ、24 時間後の細胞内における luciferase activity を測定した。

次に、AMPK-RPK の HIV-1 におよぼす影響を更に詳細に検討するため、AMPK-RPK タンパク発現レベルの異なるヒト細胞株を樹立し、その発現レベルとウイルス感染への影響度の相関性があるかどうかを解析した (図3)。

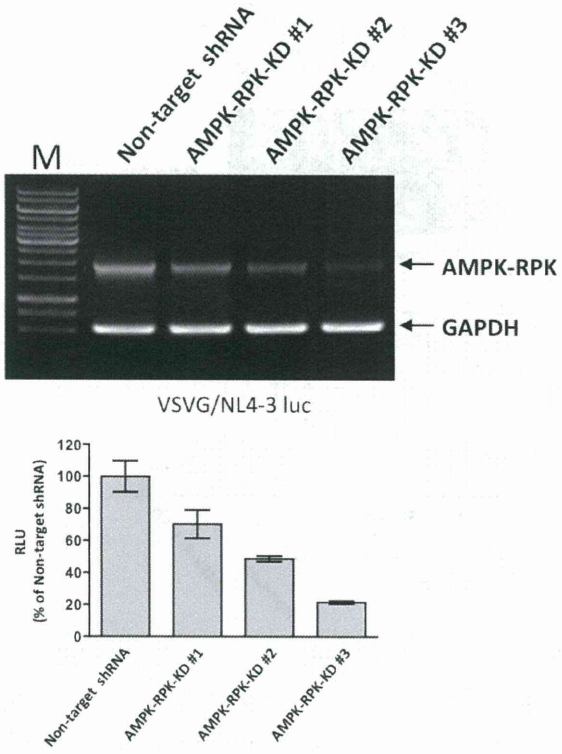

図３．AMPK-RPK 発現レベルと HIV 感染効率との相関性．上段：AMPK-RPK を標的とした shRNA を用いて様々な AMPK-RPK-KD 細胞（#1, #2, #3）を作製し、shRNA コントロール細胞（Non-target shRNA）の内在性 AMPK-RPK の mRNA 量と比較した結果。下段：MT-4/CCR5、Non-target shRNA、AMPK-RPK-KD 細胞（#1-#3）に VSVG/NL-luc HIV-1 を感染させ、24 時間後の細胞内における luciferase activity を測定した。

その結果、AMPK-RPK 発現レベルと HIV-1 感染効率との相関性が得られたことから、AMPK-RPK が HIV-1 感染制御因子であることが強く示唆された。そこで、AMPK-RPK の HIV-1 感染制御メカニズムを解析するため、まずは HIV-1 生活環の各過程における AMPK-RPK の影響について解析を進めた。具体的には、HIV-1 が標的細胞内に侵入後に起こる逆転写反応過程への影響について解析を行った。具体的には、リアルタイム PCR 法を用いて逆転写反応によって合成されるウイルス DNA 量を定量することで、この過程への影響を見極めた。その結果、AMPK-RPK-KD 細胞において、ウイルス DNA 合成効率が著しく低下していることが明らかとなった（図４）。

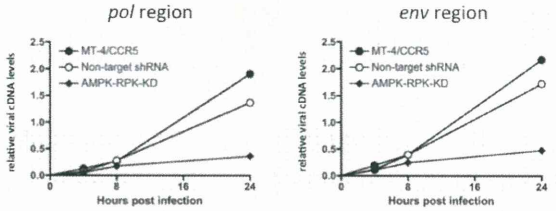

図４．AMPK-RPK の逆転写反応過程に対する影響．MT-4/CCR5、Non-target shRNA、AMPK-RPK-KD 細胞に HIV-1（NL4-3 株）を感染させ、4、8、24 時間の各時間において Total DNA を抽出し、HIV-1 の pol および env 領域を特異的に増幅するリアルタイム PCR 法を用いてウイルス DNA 合成量を定量した。

また、感染細胞内における逆転写反応が行われる場は、ウイルス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安定性に影響を及ぼしているかどうかを検討するため、Fate-of-capsid 法を用いて CA コアの安定性について解析を行った。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５）。

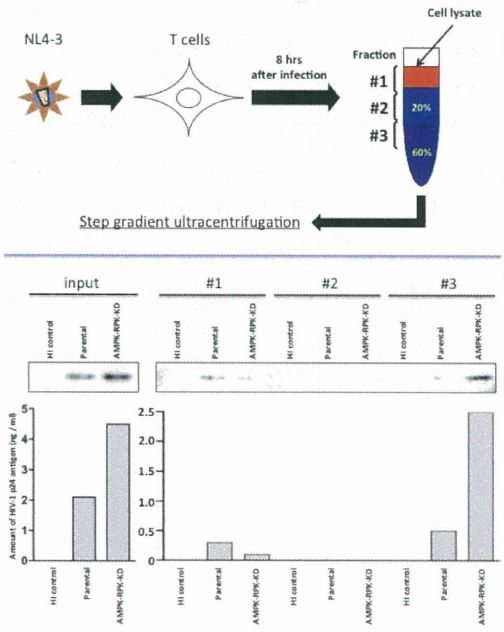

図５．HIV 感染細胞内における CA コアの安定性に対する AMPK-RPK の影響．上段：MT-4/CCR5、Non-target shRNA、AMPK-RPK-KD 細胞に HIV-1(NL4-3 株)を感染させ、8 時間後に感染細胞の細胞質分画を抽出し、20-60%シュクロースの上に細胞質分画を重層し、超遠心法にて CA コアを分離した。CA コアは#3 分画に集積している。下段：#1, #2, #3 の各分画について CA タンパクを検出する Western blotting を行った。下部のグラフは、各分画を ELISA 法にて定量を行った結果を示している。

更には、MT-4/CCR5、Non-target shRNA および AMPK-RPK-KD T 細胞株を用いた HIV-1 感染実験(multi-round infection)を行った結果、AMPK-RPK-KD T 細胞株における HIV-1 感染増殖伝播効率が顕著に低下することが明らかとなった（図５）。

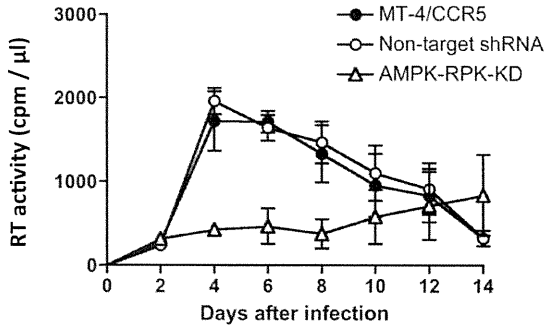

図６．HIV 感染増殖効率に対する AMPK-RPK の影響．MT-4/CCR5、Non-target shRNA、AMPK-RPK-KD 細胞に HIV-1(NL4-3 株)を感染させ、経時的に培養上清中の RT activity を測定した。

以上の結果より、AMPK-RPK 発現量が低下することで、HIV-1 感染細胞内において、CA コアの安定性に影響を与え、逆転写反応効率を低下させた結果、HIV-1 感染増殖伝播効率を低下させることが明らかとなった。よって、AMPK-RPK は HIV-1 感染必須因子であることが強く示唆された。

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２年目の研究成果については、初年度に得られた 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）HIV-1 感染制御因子候補群から単一機能遺伝子発現抑制 T 細胞株を樹立し、HIV 感染増殖効率が著しく低下している複数の細胞群（semi-clonal population）の中で、６種の shRNA が混在している細胞群について、単一 shRNA 発現 T 細胞株を６種樹立した。（２）（１）で樹立した shRNA 発現 T 細胞株を用いて HIV-1 感染実験（single-round infection）を行った結果、６種類のうち２種類の T 細胞株において感染効率の顕著な低下が認められた。この２種の宿主因子の作用機序について解析を進めたところ、１つは HIV-1 の細胞侵入時に影響を及ぼし、もう１つは HIV-1 の細胞侵入後の逆転写反応に影響をおよぼすことが明らかとなった。更には、HIV-1 感染実験（multi-round infection）の結果、この２つの遺伝子発現抑制 T 細胞株は、大元の semi-clonal population と同等の感染増殖効率の低下が認められた。また、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２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、当該研究事業２年目の目標をほぼ予定通り達成出来たと考えられる。最終年度については、前年度に同定した２種の宿主因子について重点的に詳細な解析をすすめていくと共に、HIV-1 感染耐性 shRNA 発現 T 細胞株からの HIV-1 感染制御宿主因子群の同

定作業を順次すすめていく。具体的には、HIV-1 の細胞侵入過程に影響をおよぼす宿主因子については、HIV-1 と細胞膜との融合過程の詳細な解析をおこなう。また、逆転写反応過程に影響をおよぼす宿主因子については、逆転写反応に必要な HIV-1 CA core の安定性を、当該事業初年度に手法を確立した viral core binding assay 等を用いて解析をおこなっていく。また、新たな単独 shRNA 発現 T 細胞株の評価方法として、ウイルス感染前期過程においては、luciferase 発現 HIV-1 を用いて感染性への影響を見極める。また後期過程については、ウイルス蛋白合成効率およびウイルス産生効率とを、ウイルス蛋白を ELISA 法、RT 活性もしくは western blotting 法にて検討する。さらにウイルスと宿主因子との相互作用については、免疫沈降法および免疫染色法にて検討する。

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

当該研究事業を２年間遂行した結果、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 感染制御宿主因子候補群を多数見出す事が出来た。その中で新たな HIV 感染制御機構を持つ宿主因子の存在を見出す事が出来た。

F. 健康危険情報

該当なし

G. 研究発表

1. 論文発表

1) **Takeuchi H***., Ishii H., Kuwano T., Inagaki N., Akari H., and Matano T. Host cell species-specific effect of cyclosporine A on simian immunodeficiency virus replication. *Retrovirology,* 9(1):3. 2012.

2) Sakuma R and **Takeuchi H***. Retroviral Host Cell Factors: APOBEC3G, TRIM5, and Cyclophilins. *HIV and AIDS - Updates on Biology, Immunology, Epidemiology and Treatment Strategies* ISBN 978-953-307-665-2:183-196. 2011.

3) Sugiyama R., Nishitsuji H., Furukawa A., Katahira M., Habu Y., **Takeuchi H**., Ryo A., and Takaku H. Heat shock protein 70 inhibits HIV-1 Vif-mediated ubiquitination and degradation of APOBEC3G. *Journal of Biological Chemistry* 286(12):10051-7. 2011.

4) Ohmine S., Sakuma R., Sakuma T., Thatava T., **Takeuchi H**., and Ikeda Y. The antiviral spectra of TRIM5α orthologues and human TRIM family proteins against lentiviral production. *PLoS ONE* 6(1) e16121. 2011.

*** Corresponding author**

2. 学会発表

該当なし

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（参考）

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ
Sakuma R., **Takeuchi H**	Retroviral Host Cell Factors: APOBEC3G, TRIM5, and Cyclophilins.	Nancy Dumais	***HIV and AIDS - Updates on Biology, Immunology, Epidemiology and Treatment Strategies***	Intech	Croatia	2011	ISBN 978-953-307-665-2: 183-196.

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Takeuchi H., Ishii H., Kuwano T., Inagaki N., Akari H., Matano T	Host cell species-specific effect of cyclosporine A on simian immunodeficiency virus replication.	***Retrovirology***	9(1)	3	2012
Sugiyama R., Nishitsuji H., Furukawa A., Katahira M., Habu Y., **Takeuchi H.**, Ryo A., Takaku H	Heat shock protein 70 inhibits HIV-1 Vif-mediated ubiquitination and degradation of APOBEC3G.	***Journal of Biological Chemistry***	286(12)	10051-7	2011
Ohmine S., Sakuma R., Sakuma T., Thatava T., **Takeuchi H.**, Ikeda Y	The antiviral spectra of TRIM5α orthologues and human TRIM family proteins against lentiviral production.	***PLoS ONE***	6(1)	e16121	2011

7

Retroviral Host Cell Factors: TRIM5, APOBEC3G and Cyclophilins

Ryuta Sakuma and Hiroaki Takeuchi
Department of Molecular Virology, Tokyo Medical and Dental University, Japan

1. Introduction

The conventional innate and adaptive immune systems are very effective at viral infections. However, for retroviral infections, there is another immune system that can recognize at multiple levels e.g. expression of internal host factors with antiviral activity. This is a component of viral recognition and subsequent restriction that has been called "intrinsic immunity"(Bieniasz, 2004). Intrinsic immunity can distinguish from innate and adaptive immunity, and it does not need to be induced by viral infections. Retrovirus replication has many steps in common with other retroviruses. Upon entry into the cytoplasm of target cells, some host factors are required for efficient retroviral replication cycle, and others act as restriction factors that block reverse transcription and ligation of viral cDNA to chromosomal DNA. Recently, several host factors have been identified such as the proline isomerase cyclophilin A (CypA), ApoB mRNA editing catalytic subunit (APOBEC) and tripartite motif protein 5 alpha (TRIM5α) against retrovirus infection. This review will focus on how these host factors modulate retroviral activity. It will then present our current understanding of the mechanism that may explain zoonotic transmission of retroviruses.

1.1 Fv1 and Fv4: Restriction factors that block infection by Friend-MLV in murine cells

The most intensively studied anti-cellular gene is Friend virus susceptibility (Fv) gene in laboratory mice. Fv1 and Fv4 were of special interest in Fv alleles because cultured murine cells containing them were resistant to infection by Friend murine leukemia virus (MLV)(Gardner et al., 1980; Hartley et al., 1970; Pincus et al., 1971; Rasheed and Gardner, 1983; Suzuki, 1975). Fv1-mediated restriction of MLV, for instance, is a well-studied representative of a class of restriction factors that act after membrane fusion, are highly virus-specific (Goff, 2004). Fv1 has two alleles, $Fv1^n$ and $Fv1^b$, targeting B- and N-tropic MLV, respectively (Rein et al., 1976). Fv4 was shown to encode an ecotropic MLV-like *env* gene and recent report showed that Fv4 inhibits infection by exerting dominant negative effect on MLV Env (Takeda and Matano, 2007). Although the precise mechanism of Fv1 restriction remains unclear, the important point is that the viral determinants for this type of restriction have been mapped to the capsid protein (MLV amino acid 110) and as a target of host factors that can modulate retroviral life cycle (Gautsch et al., 1978; Kozak and Chakraborti, 1996).

1.2 Ref1 and Lv1: Fv1-type restriction factors in human or primate cells

A host factor that belongs to the same category of Fv1-type restriction factors is Ref1 (restriction factor 1). Ref1 is expressed in human and other non-murine cells and imposes a similar restriction of Fv1 that is controlled by relationship between the same capsid residue (MLV CA 110) and Fv1 (Towers et al., 2000). The difference between Ref1 and Fv1 function is that Ref1 restricts retroviral replication at a step prior to reverse transcription while Fv1 seems to impose a post-reverse transcription block (Goff, 2004). Another restriction factor, lentivirus susceptibility factor 1 (Lv1), was found to be responsible for restricting HIV-1 and N-tropic MLV but not rhesus macaque simian immunodeficiency virus (SIVmac) replication in Old World monkey cells (Besnier et al., 2002; Cowan et al., 2002; Munk et al., 2002).

1.3 TRIM5α: Fv1-type host factor restricting HIV-1 in primate cells

Recently, the host protein which dictates Ref1 activity was identified as an α-isoform of rhesus macaque TRIM5α protein by the laboratory of Dr. Joseph Sodroski (Stremlau et al., 2004). TRIM5 is a member of the tripartite motif (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 primates homologues of TRIM5α were shown to explain restriction activity against retroviruses, N-MLV, and equine anemia virus (Hatziioannou et al., 2004b; Keckesova et al., 2004; Perron et al., 2004; Si et al., 2006; Song et al., 2005; Yap et al., 2004; Ylinen et al., 2005). Rhesus monkey TRIM5α has strong anti-HIV-1 activity, only modest restriction against SIVmac, and does not block MLV infection, whereas its human homologue does not active against HIV-1 infection.

TRIM5α recognizes incoming viral core, but not a monomeric capsid protein, thorough its B30.2(PRYSPRY) domain. B-box2 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 core into cytoplasm. To restrict HIV-1 infection and to recognize viral core, TRIM5α must be oligomerized through its B-box 2 and coiled-coil domains. Its RING domain has E3 ubiqutin ligase activity, and self-ubiqutination is occurred, then TRIM5α is quickly degraded. This quick degradation of TRIM5α is not necessary for post-entry restriction, since replacement of TRIM5α RING domain with the corresponding domain of TRIM21 which has lower self-ubiqutination activity and longer half life than TRIM5α didn't alter the antiviral activity. When TRIM5α was over expressed, cytoplasmic body is formed, and the cytoplasmic body is supposed to be required for its antiviral activity. During TRIM5α-mediated post-entry restriction, disassembly of viral core is induced too quickly and the accumulation of viral RT-products is reduced. MG132 treatment inhibits to induce quick-disassembly, but still HIV-1 infectivity was 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 quick-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. Recently, Pacheco *et al.* reported that new world monkey TRIM5α restricts foamy virus

infection (Pacheco et al., 2010). Another consideration is the clinical significance of TRIM5α against acquired immunodeficiency syndrome (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 (Cagliani et al., 2010; van Manen et al., 2008). Thus, TRIM5α-mediated restriction may occur multi step 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 ubiqutine-proteasome independent manner. This TRIM5α-mediated late-restriction is still controversial (Zhang et al., 2008), yet it is presumable that TRIM5α restricts HIV-1 infection and production in two distinct mechanisms. Although TRIM5α restricts HIV-1 infection in broad range of cells, its late restriction depends on a cell line (Sakuma et al., 2007).

Here is another notable class of the TRIM family called TRIM-Cyp isolated from new wold monkeys (NWM). A report from the laboratory of Dr. Jeremy Luban demonstrated that owl monkey has TRIM-Cyp that restricts HIV-1 infection (Sayah et al., 2004). Although TRIM-Cyp has a cyclophilin A 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 over-expressed rhesus TRIM-Cyp restricts HIV-1 infection and production (Brennan et al., 2008; Dietrich et al., 2010; Sakuma et al., 2010; Wilson et al., 2008).

Not like other restriction factors, the counter part of TRIM5α-mediated restrictions is not accessory gene product of HIV-1, and human TRIM5α has just a modest restriction activity. NWM cell doesn't have TRIM5α, yet even without B30.2(PRYSPRY), TRIM5-Cyp can be a defense against viral infection. These evidences suggest that TRIM5α could be a key molecule to explain the species-species barrier. And if so, TRIM5α's dual antiviral activities can block the viral transmission even from closer species like to human from monkeys.

1.4 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 HIV-1 accessory gene product, Vif (stands 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; Blanc et al., 1993; Borman et al., 1995; Fan and Peden, 1992; Gabuzda et al., 1992; Sakai et al., 1993; von Schwedler et al., 1993). Recently, some cytidine deaminases were identified as a new class of host restriction factors that target retroviruses such as HIV-1 or SIV (Cullen, 2006; Harris and Liddament, 2004). 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. Unlike TRIM5α nor Fv1, Apo3G does not exert its antiviral activity by targeting the viral capsid 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 an infection step. HIV-1 counteracts Apo3G with Vif expression. During the production of progeny virions, Vif binds to Apo3G and induces Apo3G's proteosomal degradation, resulting in the decreased steady-state levels of human Apo3G (hApo3G) (Yu et al., 2003).

There are several antiretroviral mechanisms of Apo3G against HIV-1 infection. First, Apo3G-containing virus can be resulted in a large number substitution that register as cytidine (C) to thymine (T) 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 hypermutaion'(Harris et al., 2003; Lecossier et al., 2003; Mangeat et al., 2003; Mariani et al., 2003; Yu et al., 2004; Zhang et al., 2003). Second, Apo3G can inhibit tRNA annealing or tRNA processing during reverse transcription (Guo et al., 2006; Guo et al., 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 antiretroviral activity of APOBEC family because APOBEC proteins from non-human species can also inhibit HIV-1 infection (Bishop et al., 2004a; Bishop et al., 2004b; Cullen, 2006; Mariani et al., 2003; Wiegand et al., 2004), the Vif-Apo3G interaction is thought to be species specific (Mariani et al., 2003; Simon et al., 1998). 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 (Bogerd et al., 2004; Mangeat et al., 2004; Mariani et al., 2003; 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. 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). Therefore, it is probable that SIV Vif has evolved to counteract hApo3G restriction and this might contribute zoonotic transmission of SIV.

Although the antiviral activity of Apo3G is clearly correlated with its deaminase activity (Iwatani et al., 2006; Mangeat et al., 2003; Navarro et al., 2005; Opi et al., 2006; Shindo et al., 2003; Zhang et al., 2003), some members of APOBEC family have additional anti-retrovirus activities that do not require catalytically activity of itself (Li et al., 2007; Luo et al., 2007). In fact, several reports showed that deaminase-defective Apo3G and Apo3F have antiviral activity, and some antiviral-inactive mutants of both Apo3G and Apo3F have cytidine deaminase activity (Bishop et al., 2006; Holmes et al., 2007; Newman et al., 2005; Shindo et al., 2003).

However, deaminase-defective Apo3G mutant with C288S/C291A substitutions did not show any anti-viral actibity and over-expression of the mutant could work as a dominant negative agent of wild-type Apo3G, suggesting a tightly-relationship between antiviral and deaminase activities (Miyagi et al., 2007; Opi et al., 2006). Recently, it was demonstrated that hApo3G has an intrinsic immune effect on viral DNA synthesis, which may account for cytidine deaminase-independent antiviral activity of Apo3G, and did not abort replication

steps following reverse transcription (Iwatani et al., 2007). Therefore, precise mechanism of Apo3G-dependent restriction of retroviral infection still remains unclear.

1.5 Cyclophilin A: positive factor against retrovirus replication (or restriction factor?)

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 cyclosporine A to cyclophilin A inhibits this isomerase activity (Takahashi et al., 1989). In retrovirus replication, CypA was found to bind HIV-1 capsid (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 (Bosco et al., 2002; Zhao et al., 1997), 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 cic-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 still remain unclear.

It has been well established that CypA promotes an early step of HIV-1 infection in human cells (Braaten et al., 1996a; Braaten et al., 1996c; Braaten and Luban, 2001; Franke and Luban, 1996; Franke et al., 1994; Hatziioannou et al., 2005; Sokolskaja et al., 2004; Thali et al., 1994). 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 (Ackerson et al., 1998; Braaten et al., 1996a; Braaten and Luban, 2001; Bukovsky et al., 1997; Franke et al., 1994; Ott et al., 1995; Thali et al., 1994). It is still unclear how CypA is efficiently packaged into HIV-1 virion, but several report showed that both dimerization of CA and multimerization of CypA is required for efficient binding each other (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 target cells than occurring as core assemble during HIV-1 budding from the virion producer cells, indicated that target cell CypA promotes HIV-1 infectivity (Kootstra et al., 2003; Sokolskaja et al., 2004; Towers et al., 2003).

CypA-dependent virus replication is only limited the retroviruses which encode CA that binds CypA. In fact, only those retroviruses are dependent upon CypA for replication (Braaten et al., 1996c; Franke and Luban, 1996; Franke et al., 1994; Luban et al., 1993; Thali et al., 1994). These observations suggested that CA-CypA interaction might contribute tropism determinants for retroviruses. HIV-1 infection in non-human primate cells inhibits prior to reverse transcription after virus entry (Besnier et al., 2002; Cowan et al., 2002; Hatziioannou et al., 2003; Himathongkham and Luciw, 1996; Hofmann et al., 1999; Munk et al., 2002; Shibata et al., 1995; 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 (Berthoux et al., 2004; Bukovsky et al., 1997; Cowan et al., 2002;

Dorfman and Gottlinger, 1996; Hatziioannou et al., 2004a; Hatziioannou et al., 2006; Ikeda et al., 2004; Kamada et al., 2006; Kootstra et al., 2003; Owens et al., 2004; Owens et al., 2003; Sayah et al., 2004; Shibata et al., 1991; Shibata et al., 1995; Stremlau et al., 2004; Towers et al., 2003).

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 SIV infection in human cells, and SIV Vif counteracts a CypA-imposed inhibition against SIV infection with exclusion of CypA from SIV vision (Takeuchi et al., 2007). This phenomenon could distinguish from the function of SIV Vif against hApo3G previously reported from same laboratory (Takeuchi et al., 2005) because they used human cells lacking detectable deaminase activity. This observation raised the possibility that SIV Vif is crucial for zoonotic transmission of SIV from monkey to human.

2. Conclusion

Viral replication requires a lot of host cell factors, whose species specificity may affect viral tropism. On the other hand, there exist host factors that restrict viral replication. The anti-viral 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 chapter, 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 CypA that is essential for HIV-1 replication in human cells and may affect viral tropism. Understanding of these host factors would contribute to identification of the determinants for viral tropism. Finally, understanding of the factors mediating intrinsic immunity may lead to the development of antiviral agents that can boost their potency and thereby lead to treatments for viral disease.

3. References

Ackerson, B., Rey, O., Canon, J., and Krogstad, P. (1998). Cells with high cyclophilin A content support replication of human immunodeficiency virus type 1 Gag mutants with decreased ability to incorporate cyclophilin A. *Journal of virology 72*, 303-308.

Akari, H., Sakuragi, J., Takebe, Y., Tomonaga, K., Kawamura, M., Fukasawa, M., Miura, T., Shinjo, T., and Hayami, M. (1992). Biological characterization of human immunodeficiency virus type 1 and type 2 mutants in human peripheral blood mononuclear cells. *Archives of virology 123*, 157-167.

Berthoux, L., Sebastian, S., Sokolskaja, E., and Luban, J. (2004). Lv1 inhibition of human immunodeficiency virus type 1 is counteracted by factors that stimulate synthesis or nuclear translocation of viral cDNA. *Journal of virology 78*, 11739-11750.

Besnier, C., Takeuchi, Y., and Towers, G. (2002). Restriction of lentivirus in monkeys. *Proceedings of the National Academy of Sciences of the United States of America 99*, 11920-11925.

Bieniasz, P.D. (2004). Intrinsic immunity: a front-line defense against viral attack. *Nature immunology 5*, 1109-1115.

Bishop, K.N., Holmes, R.K., and Malim, M.H. (2006). Antiviral potency of APOBEC proteins does not correlate with cytidine deamination. *Journal of virology 80*, 8450-8458.

Bishop, K.N., Holmes, R.K., Sheehy, A.M., Davidson, N.O., Cho, S.J., and Malim, M.H. (2004a). Cytidine deamination of retroviral DNA by diverse APOBEC proteins. *Curr Biol 14*, 1392-1396.

Bishop, K.N., Holmes, R.K., Sheehy, A.M., and Malim, M.H. (2004b). APOBEC-mediated editing of viral RNA. *Science* (New York, NY *305*, 645.

Blanc, D., Patience, C., Schulz, T.F., Weiss, R., and Spire, B. (1993). Transcomplementation of VIF- HIV-1 mutants in CEM cells suggests that VIF affects late steps of the viral life cycle. *Virology 193*, 186-192.

Bogerd, H.P., Doehle, B.P., Wiegand, H.L., and Cullen, B.R. (2004). A single amino acid difference in the host APOBEC3G protein controls the primate species specificity of HIV type 1 virion infectivity factor. *Proceedings of the National Academy of Sciences of the United States of America 101*, 3770-3774.

Borman, A.M., Quillent, C., Charneau, P., Dauguet, C., and Clavel, F. (1995). Human immunodeficiency virus type 1 Vif- mutant particles from restrictive cells: role of Vif in correct particle assembly and infectivity. *Journal of virology 69*, 2058-2067.

Bosco, D.A., Eisenmesser, E.Z., Pochapsky, S., Sundquist, W.I., and Kern, D. (2002). Catalysis of cis/trans isomerization in native HIV-1 capsid by human cyclophilin A. *Proceedings of the National Academy of Sciences of the United States of America 99*, 5247-5252.

Braaten, D., Aberham, C., Franke, E.K., Yin, L., Phares, W., and Luban, J. (1996a). Cyclosporine A-resistant human immunodeficiency virus type 1 mutants demonstrate that Gag encodes the functional target of cyclophilin A. *Journal of virology 70*, 5170-5176.

Braaten, D., Franke, E.K., and Luban, J. (1996b). Cyclophilin A is required for an early step in the life cycle of human immunodeficiency virus type 1 before the initiation of reverse transcription. *Journal of virology 70*, 3551-3560.

Braaten, D., Franke, E.K., and Luban, J. (1996c). Cyclophilin A is required for the replication of group M human immunodeficiency virus type 1 (HIV-1) and simian immunodeficiency virus SIV(CPZ)GAB but not group O HIV-1 or other primate immunodeficiency viruses. *Journal of virology 70*, 4220-4227.

Braaten, D., and Luban, J. (2001). Cyclophilin A regulates HIV-1 infectivity, as demonstrated by gene targeting in human T cells. *The EMBO journal 20*, 1300-1309.

Brennan, G., Kozyrev, Y., and Hu, S.L. (2008). TRIMCyp expression in Old World primates Macaca nemestrina and Macaca fascicularis. *Proceedings of the National Academy of Sciences of the United States of America 105*, 3569-3574.

Bukovsky, A.A., Weimann, A., Accola, M.A., and Gottlinger, H.G. (1997). Transfer of the HIV-1 cyclophilin-binding site to simian immunodeficiency virus from Macaca mulatta can confer both cyclosporin sensitivity and cyclosporin dependence. *Proceedings of the National Academy of Sciences of the United States of America 94*, 10943-10948.

Cagliani, R., Fumagalli, M., Biasin, M., Piacentini, L., Riva, S., Pozzoli, U., Bonaglia, M.C., Bresolin, N., Clerici, M., and Sironi, M. (2010). Long-term balancing selection maintains trans-specific polymorphisms in the human TRIM5 gene. *Hum Genet 128*, 577-588.

Colgan, J., Yuan, H.E., Franke, E.K., and Luban, J. (1996). Binding of the human immunodeficiency virus type 1 Gag polyprotein to cyclophilin A is mediated by the central region of capsid and requires Gag dimerization. *Journal of virology 70*, 4299-4310.

Cowan, S., Hatziioannou, T., Cunningham, T., Muesing, M.A., Gottlinger, H.G., and Bieniasz, P.D. (2002). Cellular inhibitors with Fv1-like activity restrict human and simian immunodeficiency virus tropism. *Proceedings of the National Academy of Sciences of the United States of America 99*, 11914-11919.

Cullen, B.R. (2006). Role and mechanism of action of the APOBEC3 family of antiretroviral resistance factors. *Journal of virology 80*, 1067-1076.

Dietrich, E.A., Jones-Engel, L., and Hu, S.L. (2010). Evolution of the antiretroviral restriction factor TRIMCyp in Old World primates. *PLoS One 5*, e14019.

Doehle, B.P., Schafer, A., and Cullen, B.R. (2005). Human APOBEC3B is a potent inhibitor of HIV-1 infectivity and is resistant to HIV-1 Vif. *Virology 339*, 281-288.

Dorfman, T., and Gottlinger, H.G. (1996). The human immunodeficiency virus type 1 capsid p2 domain confers sensitivity to the cyclophilin-binding drug SDZ NIM 811. *Journal of virology 70*, 5751-5757.

Fan, L., and Peden, K. (1992). Cell-free transmission of Vif mutants of HIV-1. Virology *190*, 19-29.

Fischer, G., Wittmann-Liebold, B., Lang, K., Kiefhaber, T., and Schmid, F.X. (1989). Cyclophilin and peptidyl-prolyl cis-trans isomerase are probably identical proteins. *Nature 337*, 476-478.

Fisher, A.G., Ensoli, B., Ivanoff, L., Chamberlain, M., Petteway, S., Ratner, L., Gallo, R.C., and Wong-Staal, F. (1987). The sor gene of HIV-1 is required for efficient virus transmission in vitro. *Science* (New York, NY *237*, 888-893.

Franke, E.K., and Luban, J. (1996). Inhibition of HIV-1 replication by cyclosporine A or related compounds correlates with the ability to disrupt the Gag-cyclophilin A interaction. *Virology 222*, 279-282.

Franke, E.K., Yuan, H.E., and Luban, J. (1994). Specific incorporation of cyclophilin A into HIV-1 virions. *Nature 372*, 359-362.

Gabuzda, D.H., Lawrence, K., Langhoff, E., Terwilliger, E., Dorfman, T., Haseltine, W.A., and Sodroski, J. (1992). Role of vif in replication of human immunodeficiency virus type 1 in CD4+ T lymphocytes. *Journal of virology 66*, 6489-6495.

Gamble, T.R., Vajdos, F.F., Yoo, S., Worthylake, D.K., Houseweart, M., Sundquist, W.I., and Hill, C.P. (1996). Crystal structure of human cyclophilin A bound to the amino-terminal domain of HIV-1 capsid. *Cell 87*, 1285-1294.

Gardner, M.B., Rasheed, S., Pal, B.K., Estes, J.D., and O'Brien, S.J. (1980). Akvr-1, a dominant murine leukemia virus restriction gene, is polymorphic in leukemia-prone wild mice. *Proceedings of the National Academy of Sciences of the United States of America 77*, 531-535.

Gautsch, J.W., Elder, J.H., Schindler, J., Jensen, F.C., and Lerner, R.A. (1978). Structural markers on core protein p30 of murine leukemia virus: functional correlation with Fv-1 tropism. *Proceedings of the National Academy of Sciences of the United States of America 75*, 4170-4174.

Goff, S.P. (2004). Genetic control of retrovirus susceptibility in mammalian cells. *Annual review of genetics 38*, 61-85.

Guo, F., Cen, S., Niu, M., Saadatmand, J., and Kleiman, L. (2006). Inhibition of formula-primed reverse transcription by human APOBEC3G during human immunodeficiency virus type 1 replication. *Journal of virology 80*, 11710-11722.

Guo, F., Cen, S., Niu, M., Yang, Y., Gorelick, R.J., and Kleiman, L. (2007). The interaction of APOBEC3G with human immunodeficiency virus type 1 nucleocapsid inhibits tRNA3Lys annealing to viral RNA. *Journal of virology 81*, 11322-11331.

Harris, R.S., Bishop, K.N., Sheehy, A.M., Craig, H.M., Petersen-Mahrt, S.K., Watt, I.N., Neuberger, M.S., and Malim, M.H. (2003). DNA deamination mediates innate immunity to retroviral infection. *Cell 113*, 803-809.

Harris, R.S., and Liddament, M.T. (2004). Retroviral restriction by APOBEC proteins. *Nat Rev Immunol 4*, 868-877.

Hartley, J.W., Rowe, W.P., and Huebner, R.J. (1970). Host-range restrictions of murine leukemia viruses in mouse embryo cell cultures. *Journal of virology 5*, 221-225.

Hatziioannou, T., Cowan, S., Goff, S.P., Bieniasz, P.D., and Towers, G.J. (2003). Restriction of multiple divergent retroviruses by Lv1 and Ref1. *The EMBO journal 22*, 385-394.

Hatziioannou, T., Cowan, S., Von Schwedler, U.K., Sundquist, W.I., and Bieniasz, P.D. (2004a). Species-specific tropism determinants in the human immunodeficiency virus type 1 capsid. *Journal of virology 78*, 6005-6012.

Hatziioannou, T., Perez-Caballero, D., Cowan, S., and Bieniasz, P.D. (2005). Cyclophilin interactions with incoming human immunodeficiency virus type 1 capsids with opposing effects on infectivity in human cells. *Journal of virology 79*, 176-183.

Hatziioannou, T., Perez-Caballero, D., Yang, A., Cowan, S., and Bieniasz, P.D. (2004b). Retrovirus resistance factors Ref1 and Lv1 are species-specific variants of TRIM5alpha. *Proceedings of the National Academy of Sciences of the United States of America 101*, 10774-10779.

Hatziioannou, T., Princiotta, M., Piatak, M., Jr., Yuan, F., Zhang, F., Lifson, J.D., and Bieniasz, P.D. (2006). Generation of simian-tropic HIV-1 by restriction factor evasion. *Science* (New York, NY *314*, 95.

Himathongkham, S., and Luciw, P.A. (1996). Restriction of HIV-1 (subtype B) replication at the entry step in rhesus macaque cells. *Virology 219*, 485-488.

Hofmann, W., Schubert, D., LaBonte, J., Munson, L., Gibson, S., Scammell, J., Ferrigno, P., and Sodroski, J. (1999). Species-specific, postentry barriers to primate immunodeficiency virus infection. *Journal of virology 73*, 10020-10028.

Holmes, R.K., Koning, F.A., Bishop, K.N., and Malim, M.H. (2007). APOBEC3F can inhibit the accumulation of HIV-1 reverse transcription products in the absence of hypermutation. Comparisons with APOBEC3G. *The Journal of biological chemistry 282*, 2587-2595.

Ikeda, Y., Ylinen, L.M., Kahar-Bador, M., and Towers, G.J. (2004). Influence of gag on human immunodeficiency virus type 1 species-specific tropism. *Journal of virology 78*, 11816-11822.

Iwatani, Y., Chan, D.S., Wang, F., Maynard, K.S., Sugiura, W., Gronenborn, A.M., Rouzina, I., Williams, M.C., Musier-Forsyth, K., and Levin, J.G. (2007). Deaminase-independent inhibition of HIV-1 reverse transcription by APOBEC3G. *Nucleic Acids Res.*

Iwatani, Y., Takeuchi, H., Strebel, K., and Levin, J.G. (2006). Biochemical activities of highly purified, catalytically active human APOBEC3G: correlation with antiviral effect. *Journal of virology 80*, 5992-6002.

Javanbakht, H., Diaz-Griffero, F., Yuan, W., Yeung, D.F., Li, X., Song, B., and Sodroski, J. (2007). The ability of multimerized cyclophilin A to restrict retrovirus infection. *Virology 367*, 19-29.

Kamada, K., Igarashi, T., Martin, M.A., Khamsri, B., Hatcho, K., Yamashita, T., Fujita, M., Uchiyama, T., and Adachi, A. (2006). Generation of HIV-1 derivatives that productively infect macaque monkey lymphoid cells. *Proceedings of the National Academy of Sciences of the United States of America 103*, 16959-16964.

Keckesova, Z., Ylinen, L.M., and Towers, G.J. (2004). The human and African green monkey TRIM5alpha genes encode Ref1 and Lv1 retroviral restriction factor activities. *Proceedings of the National Academy of Sciences of the United States of America 101*, 10780-10785.

Kootstra, N.A., Munk, C., Tonnu, N., Landau, N.R., and Verma, I.M. (2003). Abrogation of postentry restriction of HIV-1-based lentiviral vector transduction in simian cells. *Proceedings of the National Academy of Sciences of the United States of America 100*, 1298-1303.

Kozak, C.A., and Chakraborti, A. (1996). Single amino acid changes in the murine leukemia virus capsid protein gene define the target of Fv1 resistance. *Virology 225*, 300-305.

Lecossier, D., Bouchonnet, F., Clavel, F., and Hance, A.J. (2003). Hypermutation of HIV-1 DNA in the absence of the Vif protein. *Science* (New York, NY *300*, 1112.

Li, X.Y., Guo, F., Zhang, L., Kleiman, L., and Cen, S. (2007). APOBEC3G inhibits DNA strand transfer during HIV-1 reverse transcription. *The Journal of biological chemistry 282*, 32065-32074.

Liddament, M.T., Brown, W.L., Schumacher, A.J., and Harris, R.S. (2004). APOBEC3F properties and hypermutation preferences indicate activity against HIV-1 in vivo. *Curr Biol 14*, 1385-1391.

Luban, J., Bossolt, K.L., Franke, E.K., Kalpana, G.V., and Goff, S.P. (1993). Human immunodeficiency virus type 1 Gag protein binds to cyclophilins A and B. *Cell 73*, 1067-1078.

Luo, K., Wang, T., Liu, B., Tian, C., Xiao, Z., Kappes, J., and Yu, X.F. (2007). Cytidine deaminases APOBEC3G and APOBEC3F interact with human immunodeficiency virus type 1 integrase and inhibit proviral DNA formation. *Journal of virology 81*, 7238-7248.

Mangeat, B., Turelli, P., Caron, G., Friedli, M., Perrin, L., and Trono, D. (2003). Broad antiretroviral defence by human APOBEC3G through lethal editing of nascent reverse transcripts. *Nature 424*, 99-103.

Mangeat, B., Turelli, P., Liao, S., and Trono, D. (2004). A single amino acid determinant governs the species-specificsensitivity of APOBEC3G to Vif action. *The Journal of biological chemistry 279*, 14481-14483.

Mariani, R., Chen, D., Schrofelbauer, B., Navarro, F., Konig, R., Bollman, B., Munk, C., Nymark-McMahon, H., and Landau, N.R. (2003). Species-specific exclusion of APOBEC3G from HIV-1 virions by Vif. *Cell 114*, 21-31.

Mbisa, J.L., Barr, R., Thomas, J.A., Vandegraaff, N., Dorweiler, I.J., Svarovskaia, E.S., Brown, W.L., Mansky, L.M., Gorelick, R.J., Harris, R.S., *et al.* (2007). Human