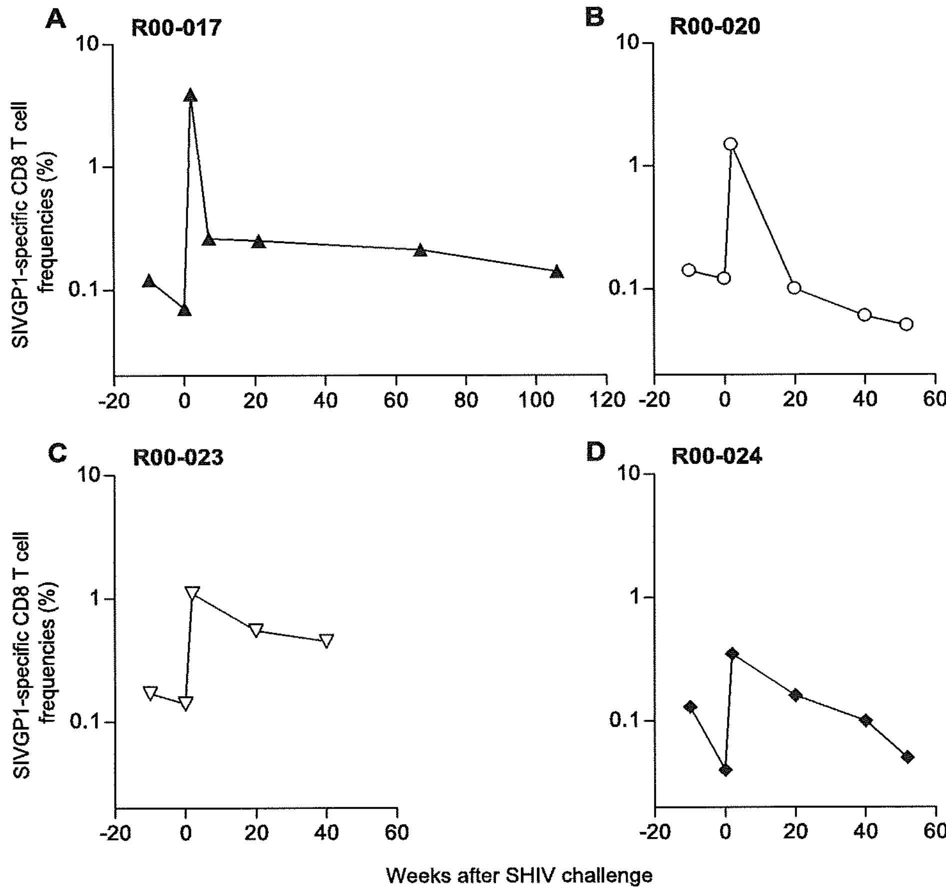


FIG. 3. SIVGP1-specific CD8^{+} T-cell frequencies in macaques before and after SHIV89.6PD challenge. Frequencies of CD8^{+} T cells showing SIVGP1-specific IFN-γ induction per total CD8^{+} T cells in PBMCs are shown. The first time point prechallenge is 10 weeks before challenge.

CD20 depletion and SIVmac239 superchallenge in the SHIV controllers. Macaques R00-017 and R00-020 were further followed up and received monoclonal anti-CD20 antibody administration at week 166 (R00-017) or week 140 (R00-020) and SIVmac239 superchallenge at week 203 (R00-017) or week 151 (R00-020) (Table 1). Viral control was not abrogated, and plasma viremia remained undetectable after anti-CD20 antibody administration (Fig. 4). In both macaques, SHIV89.6PD-specific neutralizing antibodies (NAbs) were induced efficiently after SHIV89.6PD challenge and maintained at high levels in the chronic phase (54). The monoclonal anti-CD20 antibody administration resulted in rapid and prolonged depletion of peripheral CD20^{+} lymphocytes, and more than a few months later, an approximately fourfold reduction in SHIV-specific NAb levels was observed (Fig. 5).

The following SIVmac239 superchallenge was contained in both macaques (Fig. 4). Macaque R00-017 did not show detectable plasma viremia even after SIVmac239 superchallenge, and macaque R00-020 showed only transient appearance of plasma viremia 1 week after SIVmac239 superchallenge. SIVmac239 *env* RNA but not SHIV89.6PD *env* RNA was detected in the transient plasma viremia (Fig. 6). SIVGP1-specific CD8^{+} T-cell frequencies were at marginal levels just

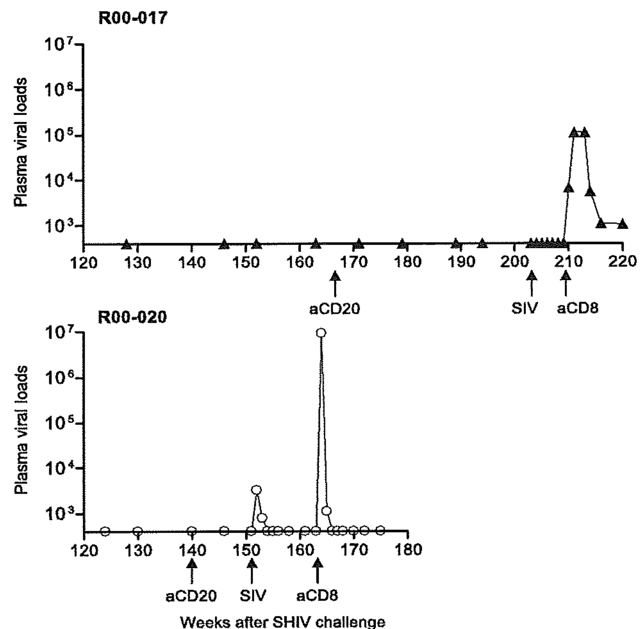


FIG. 4. Plasma viral loads (SIV *gag* RNA copies/ml plasma) in macaques R00-017 (upper panel) and R00-020 (lower panel) after week 120 post-SHIV challenge. aCD20 and aCD8, anti-CD20 and anti-CD8, respectively.

Downloaded from jvi.asm.org by on October 12, 2007

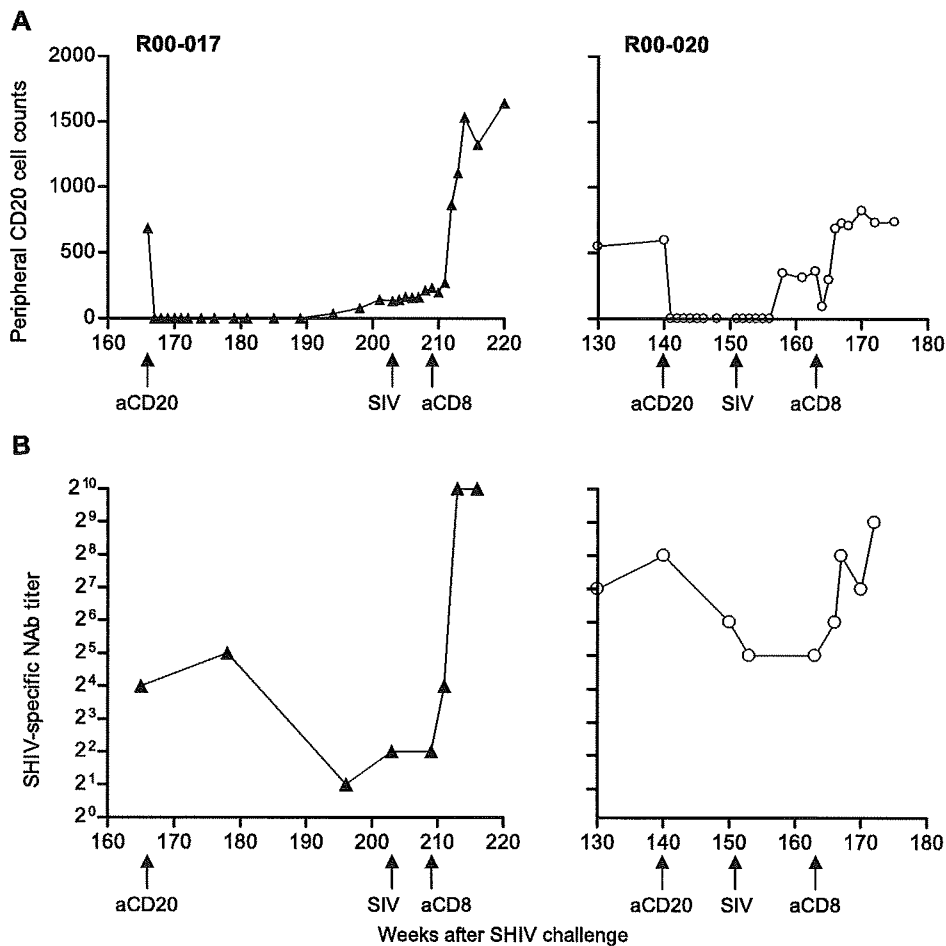


FIG. 5. Changes in SHIV89.6PD-specific NAb levels after monoclonal anti-CD20 antibody administration at week 166 in macaque R00-017 (left panels) and at week 140 in macaque R00-020 (right panels). (A) Peripheral CD20$^+$ cell counts (per μl). (B) SHIV89.6PD-specific neutralizing titers in plasma. aCD20 and aCD8, anti-CD20 and anti-CD8, respectively.

before SIVmac239 superchallenge but increased after the superchallenge (Fig. 7).

CD8 depletion after SIVmac239 superchallenge. Macaques R00-017 and R00-020 received monoclonal anti-CD8 antibody administration at week 209 (6 weeks after superchallenge) and week 163 (12 weeks after superchallenge), respectively (Table 1). Both macaques showed transient depletion of peripheral CD8$^+$ T lymphocytes and appearance of plasma viremia after the anti-CD8 antibody administration (Fig. 6).

In macaque R00-020, exhibiting a shorter period of CD8$^+$ T-lymphocyte depletion, plasma viremia was transient and detectable only at weeks 164 and 165, 1 and 2 weeks after the initial anti-CD8 antibody treatment. SIVmac239 *env* RNA but not SHIV89.6PD *env* RNA was detected in the transient plasma viremia. In macaque R00-017, exhibiting a longer period of CD8$^+$ T-lymphocyte depletion, plasma viremia appeared at week 210, 1 week after the initial anti-CD8 antibody treatment, and remained detectable during the observation period of 3 months. Interestingly, both SIVmac239 *env* RNA and SHIV89.6PD *env* RNA were detected; the former became detectable at week 210 and was detected during the observation period, whereas the latter was detected only at weeks 211 and 212. The former SIVmac239 *env* RNA levels peaked at week 213, and the latter SHIV89.6PD *env* RNA levels peaked at week 211.

SIVmac239-specific NAb responses were undetectable even after SIVmac239 superchallenge and CD8 depletion in both of the macaques (data not shown). SHIV89.6PD-specific NAb titers increased after the CD8 depletion not only in macaque R00-017 showing SHIV89.6PD viremia but also in macaque R00-020 without SHIV89.6PD viremia (Fig. 5). Both macaques showed increases in SIVGP1-specific CD8$^+$ T-cell frequencies after recovery from peripheral CD8$^+$ T-lymphocyte depletion (Fig. 7).

DISCUSSION

Previous CD8$^+$ cell depletion experiments in macaques using a monoclonal anti-CD8 antibody have indicated the importance of CD8$^+$ cell responses in SIV control in vivo (12, 29, 42). The present study evaluated the anti-SIV efficacy of these bulk CD8$^+$ cells in the vaccinated macaques that exhibited prophylactic SeV-Gag vaccine-based control of viral replication and showed induction of CD8$^+$ cells able to efficiently

Downloaded from jvi.asm.org by on October 12, 2007

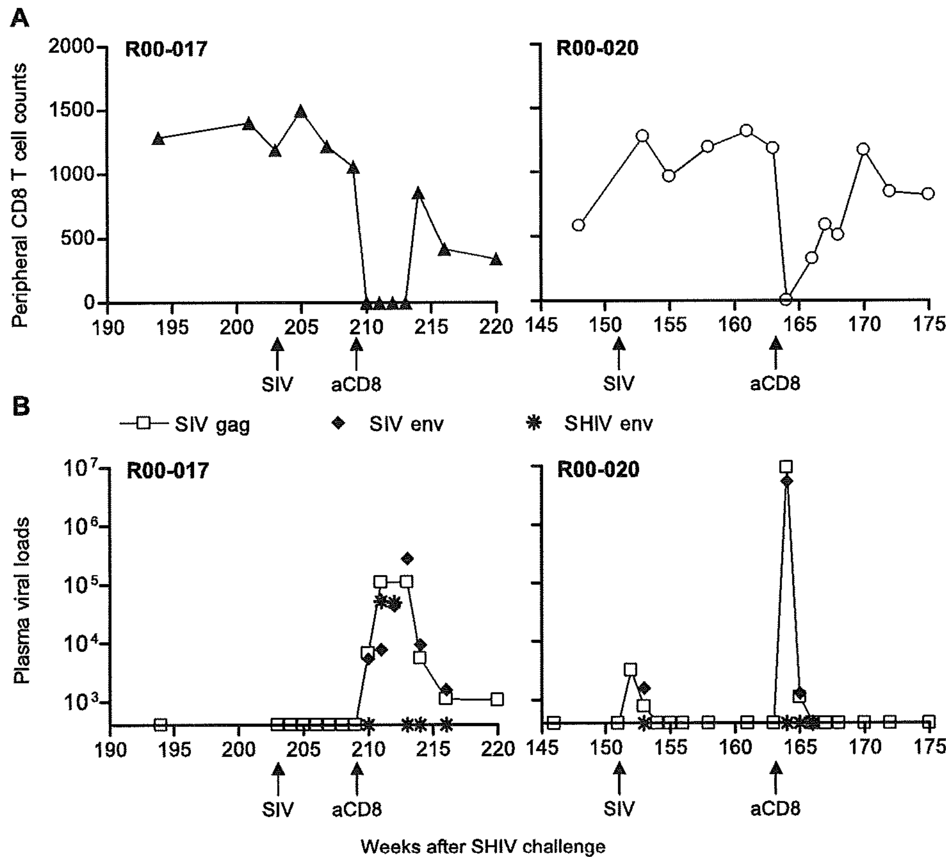


FIG. 6. SIVmac239 superchallenge and $CD8^+$ cell depletion in macaques R00-017 and R00-020. Macaque R00-017 received SIVmac239 superchallenge at week 203 and monoclonal anti-CD8 (aCD8) antibody administration starting at week 209, while macaque R00-020 received superchallenge at week 151 and anti-CD8 at week 163. (A) Peripheral $CD8^+$ T-cell counts (per μl) in macaques R00-017 (left panel) and R00-020 (right panel). (B) Plasma viral loads (copies/ml plasma) in macaques R00-017 (left panel) and R00-020 (right panel). In addition to SIV *gag* RNA levels, levels of SIV *env* RNA and SHIV *env* RNA at several time points are shown.

suppress SIV replication in vitro after SHIV challenge in these macaques. The difference in anti-SIV efficacies between postvaccination/prechallenge and postchallenge $CD8^+$ cells may explain why protective immune responses can be consistently induced not by current viral vector vaccination but by live virus infection.

These bulk $CD8^+$ cells are considered to include $CD8^+$ NK cells in addition to $CD8^+$ T lymphocytes. While previous studies using bulk $CD8^+$ cells or CTL clones (9, 24, 48, 55) have shown the importance of CTL activity on suppression of HIV/SIV replication, there may be a possibility that NK cells exert some suppressive effect on SIV replication, contributing to reductions in SIV production by prevaccination $CD8^+$ cells in the present study. The suppressive effect of postvaccination/prechallenge $CD8^+$ cells was not larger than that of prevaccination except for macaque R00-020. In contrast, postchallenge $CD8^+$ cells suppressed SIV replication more efficiently than those prevaccination and postvaccination. In the in vitro assay of SIV replication, individual macaques showed different sensitivities of target $CD8^-$ cells to SIV infection and different patterns of SIV replication kinetics in the absence of $CD8^+$ cells (Fig. 1). In macaque R00-023 showing higher levels of SIV production in the absence of $CD8^+$ cells, SIV infection at a lower MOI might exhibit a larger reduction in SIV production by addition of postchallenge $CD8^+$ cells.

Gag-specific $CD8^+$ T-cell levels peaked around 1 week after SeV-Gag vaccination and then decreased in the late phase after that (28). To prepare postvaccination/prechallenge $CD8^+$ cells, we used PBMCs in the late phase without those at week 1 post-SeV-Gag vaccination that include the peak levels of Gag-specific $CD8^+$ T cells. Thus, we compared anti-SIV efficacy of $CD8^+$ cells in the late phase postvaccination with that in the chronic phase post-SHIV challenge in this study. The postvaccination/prechallenge SIVGP1-specific $CD8^+$ T-cell frequencies roughly reflect Gag-specific $CD8^+$ T-cell ones because SIVGP1-specific $CD8^+$ T-cell responses were undetectable before SeV-Gag vaccination (data not shown). On the other hand, the postchallenge SIVGP1-specific $CD8^+$ T-cell responses are considered specific for SHIV antigens, including SIV-derived Gag, Pol, Vif, and partial Vpr. Therefore, our results shown in Fig. 3 suggest that SIV-specific $CD8^+$ T-cell frequencies in the chronic phase post-SHIV challenge were less than those post-SeV-Gag vaccination (prechallenge) in macaque R00-020. Interestingly, however, such postchallenge $CD8^+$ cells suppressed SIV replication more efficiently than postvaccination/prechallenge ones. Thus, SIV-specific $CD8^+$

Downloaded from jvi.asm.org by on October 12, 2007

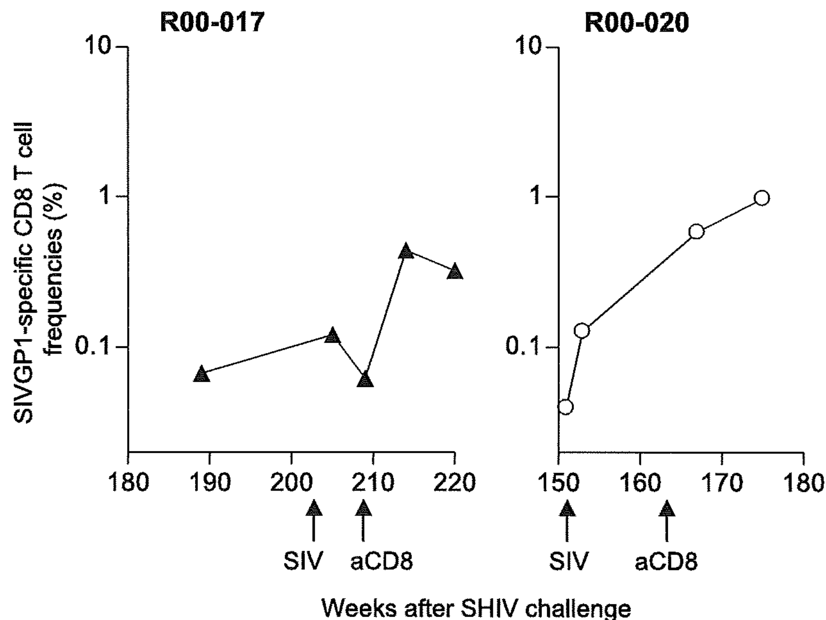


FIG. 7. SIVGP1-specific $CD8^+$ T-cell frequencies in macaques R00-017 (left panel) and R00-020 (right panel) before and after SIVmac239 superchallenge. Frequencies of $CD8^+$ T cells showing SIVGP1-specific IFN-γ induction per total $CD8^+$ T cells in PBMCs are shown. aCD8, anti-CD8.

T-cell frequencies may not always correlate with anti-SIV efficacy in vitro. It may be because postchallenge-induced, certain epitope-specific $CD8^+$ T cells may have higher anti-SIV efficacy in vitro than postvaccination/prechallenge $CD8^+$ T cells in this macaque. There may be a possibility of augmentation of anti-SIV efficacy by induction of broader $CD8^+$ T-cell responses after SHIV challenge.

A previous $CD8^+$ cell depletion study in macaques infected with live attenuated SIV has shown partial loss of superchallenged SIVmac251 control by monoclonal anti-CD8 antibody administration at the superchallenge and has suggested involvement of both cellular and humoral immune responses in this control (43). On the other hand, administration of monoclonal anti-CD8 antibody to macaques infected with live attenuated SIVmac239Δnef after SIVmac251 superchallenge resulted in the appearance of SIVmac239Δnef viremia without detectable SIVmac251 viremia (33). In contrast, the present study showed the appearance of superchallenged SIVmac239 viremia by monoclonal anti-CD8 antibody administration after superchallenge, suggesting that $CD8^+$ cells were crucial for the control of superchallenged SIVmac239 replication. It can be speculated that, in SIVmac239Δnef-infected animals, live virus replication levels before superchallenge were higher, resulting in more strict containment of superchallenge than that in our study. Additionally, neutralizing antibody responses may be involved in the containment of superchallenge in SIVmac239Δnef-infected animals but not in SHIV-infected ones. Thus, our results imply a more profound contribution of $CD8^+$ cells to control of SIV superchallenge in the absence of NAb help.

More than a few months after the anti-CD20 antibody administration, both macaques R00-017 and R00-020 showed fourfold reductions in SHIV-specific neutralizing titers, although it is unclear if these reductions were due to the $CD20^+$ cell depletion. Macaque R00-017 with a lower neutralizing titer showed transient appearance of SHIV viremia by $CD8^+$ cell depletion, but macaque R00-020 with a higher titer did not. These results were consistent with the previous study indicating involvement of humoral as well as cellular immune responses in the CXCR4-tropic SHIV control (26).

In summary, our results indicate that $CD8^+$ cells acquired the ability to efficiently suppress CCR5-tropic SIV replication in vitro by controlled CXCR4-tropic SHIV infection. While the levels of in vitro anti-SIV efficacy resulting in SIV control in vivo have not been determined, our results imply that such $CD8^+$ cell responses may be crucial for live attenuated vaccine-based containment of HIV/SIV superinfection.

ACKNOWLEDGMENTS

This work was supported by a grant from the Ministry of Education, Culture, Sports, Science, and Technology; grants from the Japan Health Sciences Foundation; and grants from the Ministry of Health, Labor, and Welfare in Japan.

The animal experiments were conducted through the Cooperative Research Program at the Tsukuba Primate Research Center, National Institute of Biomedical Innovation, with the help of the Corporation for Production and Research of Laboratory Primates. We thank Centocor, Inc., and K. A. Reimann for providing cM-T807 and M. Takiguchi, F. Ono, K. Komatsuzaki, A. Hiyaoka, A. Oyama, K. Oto, H. Akari, K. Terao, M. Miyazawa, A. Kimura, K. Mori, N. Yamamoto, T. Sata, T. Kurata, Y. Nagai, and A. Nomoto for their help.

REFERENCES

1. **Altfeld, M., and E. S. Rosenberg.** 2000. The role of $CD4^+$ T helper cells in the cytotoxic T lymphocyte response to HIV-1. Curr. Opin. Immunol. **12:** 375–380.
2. **Amara, R. R., F. Villinger, J. D. Altman, S. L. Lydy, S. P. O'Neil, S. I. Staprans, D. C. Montefiori, Y. Xu, J. G. Herndon, L. S. Wyatt, M. A.**

Downloaded from jvi.asm.org by on October 12, 2007

Candido, N. L. Kozyr, P. L. Earl, J. M. Smith, H. L. Ma, B. D. Grimm, M. L. Hulsey, J. Miller, H. M. McClure, J. M. McNicholl, B. Moss, and H. L. Robinson. 2001. Control of a mucosal challenge and prevention of AIDS in rhesus macaques by a multiprotein DNA/MVA vaccine. Science **292:**69–74.

3. **Baba, T. W., Y. S. Jeong, D. Pennick, R. Bronson, M. F. Greene, and R. M. Ruprecht.** 1995. Pathogenicity of live, attenuated SIV after mucosal infection of neonatal macaques. Science **267:**1820–1825.
4. **Borrow, P., H. Lewicki, B. H. Hahn, G. M. Shaw, and M. B. A. Oldstone.** 1994. Virus-specific CD8+ cytotoxic T-lymphocyte activity associated with control of viremia in primary human immunodeficiency virus type 1 infection. J. Virol. **68:**6103–6110.
5. **Brander, C., and B. D. Walker.** 1999. T lymphocyte responses in HIV-1 infection: implication for vaccine development. Curr. Opin. Immunol. **11:** 451–459.
6. **Casimiro, D. R., F. Wang, W. A. Schleif, X. Liang, Z.-Q. Zhang, T. W. Tobery, M.-E. Davies, A. B. McDermott, D. H. O'Connor, A. Fridman, A. Bagchi, L. G. Tussey, A. J. Bett, A. C. Finnefrock, T.-M. Fu, A. Tang, K. A. Wilson, M. Chen, H. C. Perry, G. J. Heidecker, D. C. Freed, A. Carella, K. S. Punt, K. J. Sykes, L. Huang, V. I. Ausensi, M. Bachinsky, U. Sadasivan-Nair, D. I. Watkins, E. A. Emini, and J. W. Shiver.** 2005. Attenuation of simian immunodeficiency virus SIVmac239 infection by prophylactic immunization with DNA and recombinant adenoviral vaccine vectors expressing Gag. J. Virol. **79:**15547–15555.
7. **Daniel, M. D., F. Kirchhoff, S. C. Czajak, P. K. Sehgal, and R. C. Desrosiers.** 1992. Protective effects of a live attenuated SIV vaccine with a deletion in the nef gene. Science **258:**1938–1941.
8. **Feinberg, M. B., and J. P. Moore.** 2002. AIDS vaccine models: challenging challenge viruses. Nat. Med. **8:**207–210.
9. **Gauduin, M.-C., R. L. Glickman, R. Means, and R. P. Johnson.** 1998. Inhibition of simian immunodeficiency virus (SIV) replication by CD8+ T lymphocytes from macaques immunized with live attenuated SIV. J. Virol. **72:**6315–6324.
10. **Goulder, P. J., and D. I. Watkins.** 2004. HIV and SIV CTL escape: implications for vaccine design. Nat. Rev. Immunol. **4:**630–640.
11. **Horton, H., T. U. Vogel, D. K. Carter, K. Vielhuber, D. H. Fuller, T. Shipley, J. T. Fuller, K. J. Kunstman, G. Sutter, D. C. Montefiori, V. Erfle, R. C. Desrosiers, N. Wilson, L. J. Picker, S. M. Wolinsky, C. Wang, D. B. Allison, and D. I. Watkins.** 2002. Immunization of rhesus macaques with a DNA prime/modified vaccinia virus Ankara boost regimen induces broad simian immunodeficiency virus (SIV)-specific T-cell responses and reduces initial viral replication but does not prevent disease progression following challenge with pathogenic SIVmac239. J. Virol. **76:**7187–7202.
12. **Jin, X., D. E. Bauer, S. E. Tuttleton, S. Lewin, A. Gettie, J. Blanchard, C. E. Irwin, J. T. Safrit, J. Mittler, L. Weinberger, L. G. Kostrikis, L. Zhang, A. S. Perelson, and D. D. Ho.** 1999. Dramatic rise in plasma viremia after CD8+ T cell depletion in simian immunodeficiency virus-infected macaques. J. Exp. Med. **189:**991–998.
13. **Johnson, R. P., and R. C. Desrosiers.** 1998. Protective immunity induced by live attenuated simian immunodeficiency virus. Curr. Opin. Immunol. **10:** 436–443.
14. **Johnson, R. P., J. D. Lifson, S. C. Czajak, K. S. Cole, K. H. Manson, R. Glickman, J. Yang, D. C. Montefiori, R. Montelaro, M. S. Wyand, and R. C. Desrosiers.** 1999. Highly attenuated vaccine strains of simian immunodeficiency virus protect against vaginal challenge: inverse relationship of degree of protection with level of attenuation. J. Virol. **73:**4952–4961.
15. **Kano, M., T. Matano, A. Kato, H. Nakamura, A. Takeda, Y. Suzaki, Y. Ami, K. Terao, and Y. Nagai.** 2002. Primary replication of a recombinant Sendai virus vector in macaques. J. Gen. Virol. **83:**1377–1386.
16. **Kato, A., Y. Sakai, T. Shioda, T. Kondo, M. Nakanishi, and Y. Nagai.** 1996. Initiation of Sendai virus multiplication from transfected cDNA or RNA with negative or positive sense. Genes Cells **1:**569–579.
17. **Kawada, M., T. Tsukamoto, H. Yamamoto, A. Takeda, H. Igarashi, D. I. Watkins, and T. Matano.** 2007. Long-term control of simian immunodeficiency virus replication with central memory CD4+ T-cell preservation after nonsterile protection by a cytotoxic T-lymphocyte-based vaccine. J. Virol. **81:**5202–5211.
18. **Kestler, H. W., III, D. J. Ringler, K. Mori, D. L. Panicali, P. K. Sehgal, M. D. Daniel, and R. C. Desrosiers.** 1991. Importance of the nef gene for maintenance of high virus loads and for development of AIDS. Cell **65:**651–662.
19. **Koff, W. C., P. R. Johnson, D. I. Watkins, D. R. Burton, J. D. Lifson, K. J. Hasenkrug, A. B. McDermott, A. Schultz, T. J. Zamb, R. Boyle, and R. C. Desrosiers.** 2006. HIV vaccine design: insights from live attenuated SIV vaccines. Nat. Immunol. **7:**19–23.
20. **Koup, R. A., J. T. Safrit, Y. Cao, C. A. Andrews, G. McLeod, W. Borkowsky, C. Farthing, and D. D. Ho.** 1994. Temporal association of cellular immune responses with the initial control of viremia in primary human immunodeficiency virus type 1 syndrome. J. Virol. **68:**4650–4655.
21. **Letvin, N. L., J. R. Mascola, Y. Sun, D. A. Gorgone, A. P. Buzby, L. Xu, Z. Y. Yang, B. Chakrabarti, S. S. Rao, J. E. Schmitz, D. C. Montefiori, B. R. Barker, F. L. Bookstein, and G. J. Nabel.** 2006. Preserved CD4+ central memory T cells and survival in vaccinated SIV-challenged monkeys. Science **312:**1530–1533.
22. **Li, H.-O., Y.-F. Zhu, M. Asakawa, H. Kuma, T. Hirata, Y. Ueda, Y.-S. Lee, M. Fukumura, A. Iida, A. Kato, Y. Nagai, and M. Hasegawa.** 2000. A cytoplasmic RNA vector derived from nontransmissible Sendai virus with efficient gene transfer and expression. J. Virol. **74:**6564–6569.
23. **Li, Q., L. Duan, J. D. Estes, Z. M. Ma, T. Rourke, Y. Wang, C. Reilly, J. Carlis, C. J. Miller, and A. T. Haase.** 2005. Peak SIV replication in resting memory CD4+ T cells depletes gut lamina propria CD4+ T cells. Nature **434:**1148–1152.
24. **Loffredo, J. T., E. G. Rakasz, J. P. Giraldo, S. P. Spencer, K. K. Grafton, S. R. Martin, G. Napoé, L. J. Yant, N. A. Wilson, and D. I. Watkins.** 2005. Tat_{28-35}SL8-specific CD8+ T lymphocytes are more effective than $Gag_{181-189}$CM9-specific CD8+ T lymphocytes at suppressing simian immunodeficiency virus replication in a functional in vitro assay. J. Virol. **79:**14986–14991.
25. **Lu, Y., C. D. Pauza, X. Lu, D. C. Montefiori, and C. J. Miller.** 1998. Rhesus macaques that become systemically infected with pathogenic SHIV 89.6-PD after intravenous, rectal, or vaginal inoculation and fail to make an antiviral antibody response rapidly develop AIDS. J. Acquir. Immune Defic. Syndr. Hum. Retrovirol. **19:**6–18.
26. **Mao, H., B. A. P. Lafont, T. Igarashi, Y. Nishimura, C. Brown, V. Hirsch, A. Buckler-White, R. Sadjadpour, and M. A. Martin.** 2005. CD8+ and CD20+ lymphocytes cooperate to control acute simian immunodeficiency virus/human immunodeficiency virus chimeric virus infections in rhesus monkeys: modulation by major histocompatibility complex genotype. J. Virol. **79:**14887–14898.
27. **Matano, T., M. Kano, H. Nakamura, A. Takeda, and Y. Nagai.** 2001. Rapid appearance of secondary immune responses and protection from acute CD4 depletion after a highly pathogenic immunodeficiency virus challenge in macaques vaccinated with a DNA prime/Sendai viral vector boost regimen. J. Virol. **75:**11891–11896.
28. **Matano, T., M. Kobayashi, H. Igarashi, A. Takeda, H. Nakamura, M. Kano, C. Sugimoto, K. Mori, A. Iida, T. Hirata, M. Hasegawa, T. Yuasa, M. Miyazawa, Y. Takahashi, M. Yasunami, A. Kimura, D. H. O'Connor, D. I. Watkins, and Y. Nagai.** 2004. Cytotoxic T lymphocyte-based control of simian immunodeficiency virus replication in a preclinical AIDS vaccine trial. J. Exp. Med. **199:**1709–1718.
29. **Matano, T., R. Shibata, C. Siemon, M. Connors, H. C. Lane, and M. A. Martin.** 1998. Administration of an anti-CD8 monoclonal antibody interferes with the clearance of chimeric simian/human immunodeficiency virus during primary infections of rhesus macaques. J. Virol. **72:**164–169.
30. **Mattapallil, J. J., D. C. Douek, A. Buckler-White, D. C. Montefiori, N. L. Letvin, G. J. Nabel, and M. Roederer.** 2006. Vaccination preserves CD4 memory T cells during acute simian immunodeficiency virus challenge. J. Exp. Med. **203:**1533–1541.
31. **Mattapallil, J. J., D. C. Douek, B. Hill, Y. Nishimura, M. A. Martin, and M. Roederer.** 2005. Massive infection and loss of memory CD4+ T cells in multiple tissues during acute SIV infection. Nature **434:**1093–1097.
32. **McMichael, A. J., and T. Hanke.** 2003. HIV vaccines 1983–2003. Nat. Med. **9:**874–880.
33. **Metzner, K. J., X. Jin, F. V. Lee, A. Gettie, D. E. Bauer, M. D. Mascio, A. S. Perelson, P. A. Marx, D. D. Ho, L. G. Kostrikis, and R. I. Connor.** 1999. Effects of in vivo CD8+ T cell depletion on virus replication in rhesus macaques immunized with a live, attenuated simian immunodeficiency virus vaccine. J. Exp. Med. **191:**1921–1932.
34. **Miller, C. J., and K. Abel.** 2005. Immune mechanisms associated with protection from vaginal SIV challenge in rhesus monkeys infected with virulence-attenuated SHIV 89.6. J. Med. Primatol. **34:**271–281.
35. **Miller, C. J., M. B. McChesney, X. Lü, P. J. Dailey, C. Chutkowski, D. Lu, P. Brosio, B. Roberts, and Y. Lu.** 1997. Rhesus macaques previously infected with simian/human immunodeficiency virus are protected from vaginal challenge with pathogenic SIVmac239. J. Virol. **71:**1911–1921.

35a. **National Institute of Infectious Diseases.** 2007. Guides for animal experiments performed at National Institute of Infectious Diseases. National Institute of Infectious Diseases, Tokyo, Japan. (In Japanese).

36. **Nishimura, Y., C. R. Brown, J. J. Mattapallil, T. Igarashi, A. Buckler-White, B. A. Lafont, V. M. Hirsch, M. Roederer, and M. A. Martin.** 2005. Resting naive CD4+ T cells are massively infected and eliminated by X4-tropic simian-human immunodeficiency viruses in macaques. Proc. Natl. Acad. Sci. USA **102:**8000–8005.
37. **Nishimura, Y., T. Igarashi, O. K. Donau, A. Buckler-White, C. Buckler, B. A. Lafont, R. M. Goeken, S. Goldstein, V. M. Hirsch, and M. A. Martin.** 2004. Highly pathogenic SHIVs and SIVs target different CD4+ T cell subsets in rhesus monkeys, explaining their divergent clinical courses. Proc. Natl. Acad. Sci. USA **101:**12324–12329.
38. **Ogg, G. S., X. Jin, S. Bonhoeffer, P. R. Dunbar, M. A. Nowak, S. Monard, J. P. Segal, Y. Cao, S. L. Rowland-Jones, V. Cerundolo, A. Hurley, M. Markowitz, D. D. Ho, D. F. Nixon, and A. J. McMichael.** 1998. Quantitation of HIV-1-specific cytotoxic T lymphocytes and plasma load of viral RNA. Science **279:**2103–2106.
39. **Picker, L. J., and D. I. Watkins.** 2005. HIV pathogenesis: the first cut is the deepest. Nat. Immunol. **6:**430–432.
40. **Rose, N. F., P. A. Marx, A. Luckay, D. F. Nixon, W. J. Moretto, S. M. Donahoe, D. Montefiori, A. Roberts, L. Buonocore, and J. K. Rose.** 2001. An

Downloaded from jvi.asm.org by on October 12, 2007

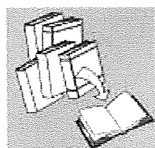
effective AIDS vaccine based on live attenuated vesicular stomatitis virus recombinants. Cell **106**:539–549.

41. **Rosenberg, E. S., J. M. Billingsley, A. M. Caliendo, S. L. Boswell, P. E. Sax, S. A. Kalams, and B. D. Walker.** 1997. Vigorous HIV-1-specific $CD4^+$ T cell responses associated with control of viremia. Science **278**:1447–1450.
42. **Schmitz, J. E., M. J. Kuroda, S. Santra, V. G. Sasseville, M. A. Simon, M. A. Lifton, P. Racz, K. Tenner-Racz, M. Dalesandro, B. J. Scallon, J. Ghrayeb, M. A. Forman, D. C. Montefiori, E. P. Rieber, N. L. Letvin, and K. A. Reimann.** 1999. Control of viremia in simian immunodeficiency virus infection by CD8+ lymphocytes. Science **283**:857–860.
43. **Schmitz, J. E., R. P. Johnson, H. M. McClure, K. H. Manson, M. S. Wyand, M. J. Kuroda, M. A. Lifton, R. S. Khunkhun, K. J. McEvers, J. Gillis, M. Piatak, J. D. Lifson, G. Grosschupff, P. Racz, K. Tenner-Racz, E. P. Rieber, K. Kuus-Reichel, R. S. Gelman, N. L. Letvin, D. C. Montefiori, R. M. Ruprecht, R. C. Desrosiers, and K. A. Reimann.** 2005. Effect of $CD8^+$ lymphocyte depletion on virus containment after simian immunodeficiency virus SIVmac251 challenge of live attenuated SIVmac239Δ3-vaccinated rhesus macaques. J. Virol. **79**:8131–8141.
44. **Shibata, R., C. Siemon, S. C. Czajak, R. C. Desrosiers, and M. A. Martin.** 1997. Live, attenuated simian immunodeficiency virus vaccines elicit potent resistance against a challenge with a human immunodeficiency virus type 1 chimeric virus. J. Virol. **71**:8141–8148.
45. **Shibata, R., F. Maldarelli, C. Siemon, T. Matano, M. Parta, G. Miller, T. Fredrickson, and M. A. Martin.** 1997. Infection and pathogenicity of chimeric simian-human immunodeficiency viruses in macaques: determinants of high virus loads and CD4 cell killing. J. Infect. Dis. **176**:362–373.
46. **Shiver, J. W., T. M. Fu, L. Chen, D. R. Casimiro, M. E. Davies, R. K. Evans, Z. Q. Zhang, A. J. Simon, W. L. Trigona, S. A. Dubey, L. Huang, V. A. Harris, R. S. Long, X. Liang, L. Handt, W. A. Schleif, L. Zhu, D. C. Freed, N. V. Persaud, L. Guan, K. S. Punt, A. Tang, M. Chen, K. A. Wilson, K. B. Collins, G. J. Heidecker, V. R. Fernandez, H. C. Perry, J. G. Joyce, K. M. Grimm, J. C. Cook, P. M. Keller, D. S. Kresock, H. Mach, R. D. Troutman, L. A. Isopi, D. M. Williams, Z. Xu, K. E. Bohannon, D. B. Volkin, D. C. Montefiori, A. Miura, G. R. Krivulka, M. A. Lifton, M. J. Kuroda, J. E. Schmitz, N. L. Letvin, M. J. Caulfield, A. J. Bett, R. Youil, D. C. Kaslow, and E. A. Emini.** 2002. Replication-incompetent adenoviral vaccine vector elicits effective anti-immunodeficiency-virus immunity. Nature **415**:331–335.
47. **Takeda, A., H. Igarashi, H. Nakamura, M. Kano, A. Iida, T. Hirata, M. Hasegawa, Y. Nagai, and T. Matano.** 2003. Protective efficacy of an AIDS vaccine, a single DNA priming followed by a single booster with a recombinant replication-defective Sendai virus vector, in a macaque AIDS model. J. Virol. **77**:9710–9715.
48. **Tomiyama, H., M. Fujiwara, S. Oka, and M. Takiguchi.** 2005. Epitope-dependent effect of Nef-mediated HLA class I down-regulation on ability of HIV-1-specific CTLs to suppress HIV-1 replication. J. Immunol. **174**:36–40.
49. **Veazey, R. S., K. G. Mansfield, I. C. Tham, A. C. Carville, D. E. Shvetz, A. E. Forand, and A. A. Lackner.** 2000. Dynamics of CCR5 expression by $CD4^+$ T cells in lymphoid tissues during simian immunodeficiency virus infection. J. Virol. **74**:11001–11007.
50. **Veazey, R. S., M. DeMaria, L. V. Chalifoux, D. E. Shvetz, D. R. Pauley, H. L. Knight, M. Rosenzweig, R. P. Johnson, R. C. Desrosiers, and A. A. Lackner.** 1998. Gastrointestinal tract as a major site of CD4+ T cell depletion and viral replication in SIV infection. Science **280**:427–431.
51. **Voss, G., S. Nick, C. Stahl-Hennig, K. Ritter, and G. Hunsmann.** 1992. Generation of macaque B lymphoblastoid cell lines with simian Epstein-Barr-like viruses: transformation procedure, characterization of the cell lines and occurrence of simian foamy virus. J. Virol. Methods **39**:185–195.
52. **Wilson, N. A., J. Reed, G. S. Napoe, S. Piaskowski, A. Szymanski, J. Furlott, E. J. Gonzalez, L. J. Yant, N. J. Maness, G. E. May, T. Soma, M. R. Reynolds, E. Rakasz, R. Rudersdorf, A. B. McDermott, D. H. O'Connor, T. C. Friedrich, D. B. Allison, A. Patki, L. J. Picker, D. R. Burton, J. Lin, L. Huang, D. Patel, G. Heindecker, J. Fan, M. Citron, M. Horton, F. Wang, X. Liang, J. W. Shiver, D. R. Casimiro, and D. I. Watkins.** 2006. Vaccine-induced cellular immune responses reduce plasma viral concentrations after repeated low-dose challenge with pathogenic simian immunodeficiency virus SIVmac239. J. Virol. **80**:5875–5885.
53. **Wyand, M. S., K. H. Manson, M. Garcia-Moll, D. C. Montefiori, and R. C. Desrosiers.** 1996. Vaccine protection by a triple deletion mutant of simian immunodeficiency virus. J. Virol. **70**:3724–3733.
54. **Yamamoto, H., M. Kawada, T. Tsukamoto, A. Takeda, H. Igarashi, M. Miyazawa, T. Naruse, M. Yasunami, A. Kimura, and T. Matano.** 2007. Vaccine-based, long-term, stable control of simian/human immunodeficiency virus 89.6PD replication in rhesus macaques. J. Gen. Virol. **88**:652–659.
55. **Yang, O. O., S. A. Kalams, A. Trocha, H. Cao, A. Luster, R. P. Johnson, and B. D. Walker.** 1997. Suppression of human immunodeficiency virus type 1 replication by $CD8^+$ cells: evidence for HLA class I-restricted triggering of cytolytic and noncytolytic mechanisms. J. Virol. **71**:3120–3128.

Downloaded from jvi.asm.org by on October 12, 2007

Rev. Med. Virol. 2008; **18**: 261–275.
Published online 2 April 2008 in Wiley InterScience
(www.interscience.wiley.com)
Reviews in Medical Virology
DOI: 10.1002/rmv.576

REVIEW



Species barrier of HIV-1 and its jumping by virus engineering

Masako Nomaguchi, Naoya Doi, Kazuya Kamada and Akio Adachi*
Department of Virology, Institute of Health Biosciences, The University of Tokushima Graduate School, Tokushima 770-8503, Japan

SUMMARY

Monkey infection models are absolutely necessary for studies of human immunodeficiency virus type 1 (HIV-1) pathogenesis and of developing drugs/vaccines against HIV-1. In addition, currently unknown roles of its accessory proteins for *in vivo* replication await elucidation by experimental approaches. Due to the fact that HIV-1 is tropic only for chimpanzees and humans, studies of this line have been impeded for a long time, although various investigations have been carried out utilising genetically related SIV and SIV/HIV chimeric virus (SHIV) as pathogens. Recent findings of anti-HIV-1 innate factors such as tripartite motif protein 5α (TRIM5α) and APOBEC3G/F prompted us to re-initiate an old and vital research project which would, as a result, confer the capability to overcome the species barrier on the HIV-1. We currently have obtained, by virus engineering through genetic manipulation and adaptation, some new and promising HIV-1 clones for *in vivo* studies in macaque monkeys as mentioned above. In this review, we summarise the past, present and future of HIV-1/SIV chimeric viruses with special reference to relevant basic HIV-1/SIV studies. Copyright © 2008 John Wiley & Sons, Ltd.

Received: 14 February 2008; Accepted: 25 February 2008

INTRODUCTION

Human immunodeficiency virus type 1 (HIV-1) productively infects only humans and chimpanzees but not Old World monkeys, and is specifically pathogenic to humans causing AIDS and AIDS-associated diseases. This narrow host range of HIV-1 has compelled us to use SIV or SHIV, a chimera between HIV-1 and SIV, as input viruses for *in vivo* model studies in macaque monkeys. Although SIV isolated from rhesus monkeys (SIVmac) is similar to HIV-1 in its genome organisation and pathogenic potential, it is a genetically and virologically distinct virus from HIV-1 in a number of important points. The same is quite true for various SHIVs that are basically SIVmac derivatives carrying only a portion of HIV-1 genome sequence [1–6]. Of note, HIV-1 genome contains a unique set of accessory genes that are believed to play essential functional roles for virus persistence, spread and pathogenesis in natural target cells and/or in individuals by modulating and optimising viral replication.

It is now well appreciated that many mammalian species including primates encode factors conferring resistance to retroviral infections. In fact, human/simian tripartite motif protein 5α (TRIM5α) and apolipoprotein B mRNA-editing enzyme-catalytic polypeptide-like 3G (APOBEC3G) (A3G) have been identified as such factors and demonstrated to have strong anti-HIV-1 activity. Importantly, the genomic regions of HIV-1 that are critical for the restriction of viral replication are also determined. Based on these studies, we could have designed a novel chimeric virus totally different from the pre-existing SHIVs, and actually created it *in vitro*. The resultant chimeric virus belongs to the HIV-1 group by standard scientific criteria and has been demonstrated to represent

*Corresponding author: Akio Adachi, Department of Virology, Institute of Health Biosciences, The University of Tokushima Graduate School, Tokushima 770-8503, Japan.
E-mail: adachi@basic.med.tokushima-u.ac.jp

Abbreviations used
HIV-1, human immunodeficiency virus type 1; SHIV, SIV/HIV chimeric virus; TRIM5α, tripartite motif protein 5α; A3G, apolipoprotein B mRNA-editing enzyme-catalytic polypeptide-like 3G (APOBEC3G); A3F, APOBEC3F; CA, capsid protein; N-MLV, N-tropic murine leukaemia virus; EIAV, equine infectious anaemia virus; CypA, cyclophilin A; CyM, cynomolgus monkey; GFP, green fluorescent protein

Copyright © 2008 John Wiley & Sons, Ltd.

Table 1. Primates and their immunodeficiency viruses

	Primate species	Virus designation	Virus grouping	Pathogenicity
Apes	Human	HIV-1	HIV-1/SIVcpz	+
		HIV-2	SIVmac/SIVsmm/HIV-2	+
	Chimpanzee	SIVcpz	HIV-1/SIVcpz	−
Old World monkeys	Macaque			
	Rhesus monkey	SIVmac	SIVmac/SIVsmm/HIV-2	+
	Cynomolgus monkey	SIVcyn		
	Pig-tailed monkey	SIVmne		
	Stamp tail monkey	SIVstm		
	Sooty mangabey	SIVsmm	SIVmac/SIVsmm/HIV-2	−
	African green monkey	SIVagm	SIVagm	−
	Mandrill	SIVmnd	SIVmnd-1 SIVmnd-2	−

Immunodeficiency viruses isolated from various primate species are classified by their genome organisation and/or nucleotide sequence homology (>90%). HIV-1 is a unique primate lentivirus. For details, see Reference [137]. (+) and (−) in the pathogenicity column indicate that the virus can induce AIDS in individuals, or not, respectively.

the first and prototype monkey-tropic HIV-1. However, the virus was found to grow less efficiently in macaque cells relative to the standard pathogenic clone SIVmac239. We are, therefore, now generating a second generation of monkey-tropic HIV-1s with positive results by virus engineering through recombinant DNA techiniques and virus adaptation in cells. In this review, we describe our basic research on chimeric viruses as well as the related virological topics.

VIROLOGICAL PROPERTIES OF SIVmac AND HIV-1

SIV was initially isolated from a rhesus monkey as an infectious agent to induce an AIDS-like disease [7,8]. Molecular clones of the virus (SIVmac) were then generated and shown to be capable of causing an AIDS-like disease in the monkeys [9–11]. Subsequently, species-specific SIVs were isolated from various monkeys including sooty mangabeys (SIVsmm) [12–14], African green monkeys (SIVagm) [15–17] and mandrill (SIVmnd) [18,19]. These SIVs establish asymptomatic chronic infections and do not develop any disease in their natural hosts (Table 1). It has been suggested that SIVmac emerged by a cross-species infection of the rhesus macaque with SIVsmm naturally found in sooty mangabeys [12,13,20]. SIVmac is similar to HIV-1 in genomic organisation (Figure 1) and in biology including pathogenicity *in vivo* [6]. Both viruses target CD4+ cells such as T-lymphocytes and macrophages, and use CCR5 as a co-receptor, resulting in the complete loss of CD4+ T-cells. They elicit persistent replication, chronic disease and eventually immunodeficiency. The infection of macaque monkeys with SIVmac is widely used as a model for HIV/AIDS to study disease progression and virus transmission.

However, some significant genetic and biological differences do exist between these two viruses. Both HIV-1 and SIVmac possess four accessory proteins, but HIV-1 can be distinguished from SIVmac by the presence and absence of Vpu and Vpx, respectively. Since it has been shown that the accessory proteins are, in many cases, dispensable for viral replication *in vitro*, the precise roles of these proteins for viral replication and pathogenesis need to be elucidated by *in vivo* study. In addition, SIVmac and HIV-1 show only 30–50% homology of the amino acid sequences, resulting in some functional differences of HIV-1 proteins and their SIVmac counterparts. First, SIVmac exhibits sensitivities to antiviral drugs that are not observed for HIV-1 [1–3]. Second, although simian and human AIDS are pathologically similar, the disease course of SIVmac is short relative to that of HIV-1 infection. SIVmac induces the immunodeficiency in individuals in 1–3 years versus

Copyright © 2008 John Wiley & Sons, Ltd.

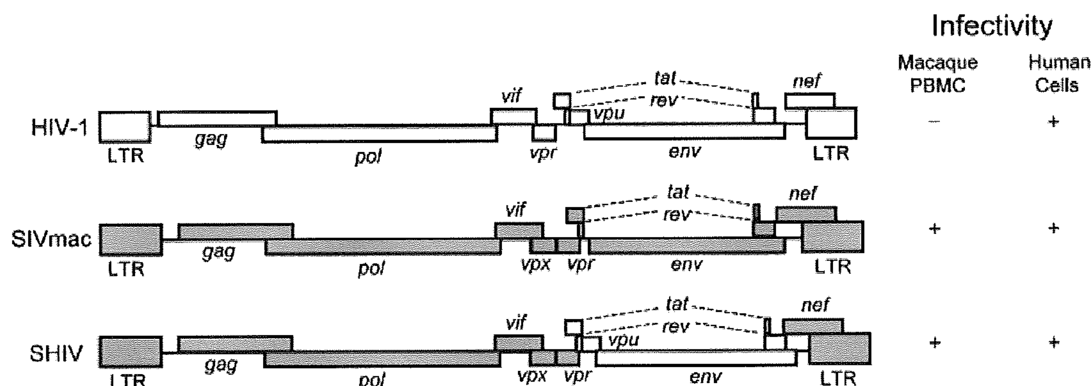


Figure 1. Genomic organisation and cellular tropism of HIV-1, SIVmac and a standard SHIV. White and grey boxes represent sequences of HIV-1 and SIVmac, respectively. (+) and (–) on the right indicate growth-competence and -incompetence, respectively

10 years on average for HIV-1 [6]. In particular, an accelerated disease progression is observed in some rhesus macaques inoculated with pathogenic strains of SIVmac and SIVsmm. In these cases, infected rhesus macaques fail to elicit immune responses and develop disease in less than 6 months [6,21,22]. This may be due to distinctive cellular and humoral immune responses that are SIV specific. Furthermore, the host cell tropism is quite different between HIV-1 and SIVmac. HIV-1 replicates in cells of humans and chimpanzees, but not in cells of Old World monkeys, such as the rhesus monkey and African green monkey, whereas SIVmac can establish productive infection in both human and monkey cells (Figure 1) [23–26]. Because of these differences between the two viruses, SIVmac macaque monkey models may restrict the utility for studies on the disease progression, the significance of HIV-1 accessory proteins for pathogenesis and the development of vaccine/drug specific for HIV-1.

GENERATION AND *IN VIVO* CHARCTERISATION OF SIVmac-DERIVED SHIV CLONES

In order to overcome the limitations of SIVmac-monkey models and to generate models that would more closely reflect HIV-1 infection and disease progression, various SHIVs have been designed and constructed. The first generation of SHIV contained HIV-1 *tat*, *rev*, *vpu* and *env* in the genetic backbone of SIVmac (Figure 1) [23,27]. This SHIV clone was shown to infect and induce immunologic responses in macaques, but did not cause AIDS-like disease. The pathogenic SHIV was obtained later by serial animal-to-animal passages of infected blood and bone marrow or by depletion of CD8+ T-cells in infected monkeys [28–31]. Macaques infected with SHIVs have been used to determine the effect of neutralising antibodies against, particularly, HIV-1 Env to prevent SHIV infection [32,33], for the evaluation of antiviral therapy and for the analysis of drug resistance emergence [2,34]. In contrast to the disease progressions observed for SIV in macaques (1–3 years) and HIV-1 in humans (10 years), SHIV cause a rapid, systemic complete loss of CD4+ T-lymphocytes within several weeks of inoculation in unvaccinated animals. Despite the rapid disease progression by SHIV, SHIV has been shown to be controlled easily by vaccines [35–37], whereas it is difficult to control SIV replication by vaccines [38–40]. It has been suggested that the rapid disease progression in SHIV-infected animals may be due to the difference in chemokine receptor usage by SHIVs and SIVs routinely employed in these experiments. SIVs predominantly use CCR5 as a coreceptor (R5-tropic), which is expressed on memory CD4+ T-lymphocytes. SIV infection induces massive loss of memory CD4+ T-lymphocytes, particularly in the gastrointestinal tract [41–43]. In contrast, SHIVs use the CXCR4 coreceptor (X4-tropic), which is expressed on naïve CD4+T-cells, for infection. X4-tropic SHIVs target naïve CD4+ T-cells and cause depletion of naïve CD4+ T-cells. The loss of naïve CD4+ T-cells results in the abrogation of any CD4+ T-cells renewal, causing a rapid and complete depletion of CD4+ T-cells in infected macaques [4,5]. In this regard,

Copyright © 2008 John Wiley & Sons, Ltd.

DOI: 10.1002/rmv

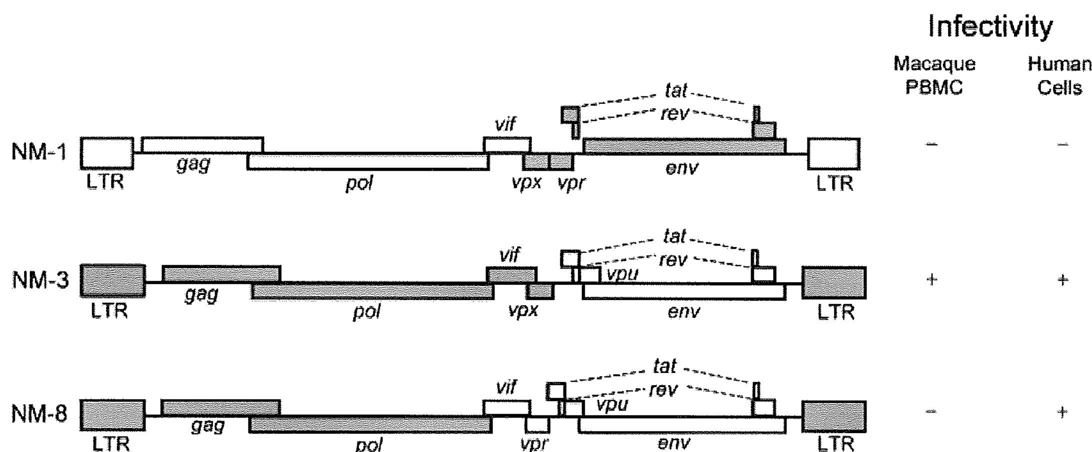


Figure 2. Genomic organisation and cellular tropism of SIVmac/HIV-1 chimeric viruses. White and grey boxes represent sequences from the parental clones NL4-3 (HIV-1) and MA239 (SIVmac), respectively. (+) and (−) on the right indicate growth-competence and -incompetence, respectively

R5-tropic pathogenic SHIV strains have been made by replacing the CXCR4-Env with CCR5-Env [44–46]. Because most primary isolates of SIVs and HIV-1 are R5-tropic, R5-ropic SHIV strains may be more relevant to HIV-1 infection and have been used for the evaluation of vaccine regimens [47,48] and for the studies of disease progression [49,50].

INNATE ANTI-HIV-1 FACTORS IN MONKEY CELLS

Early studies on viral determinants for species tropism

In order to search viral determinants that restrict the replication of HIV-1 in macaque PBMC, several SIVmac-HIV-1 chimeric viruses were constructed (Figure 2) [23,27]. Among them, only NM-3 exhibited infectivity to macaque PBMC. Genome comparison of NM-1 with NM-3 revealed that the determinant(s) of macaque cell tropism resides in the 5′-genomic region of SIVmac, since NM-1 containing the 3′-genomic region of SIVmac (*vpx*, *vpr*, *tat*, *rev* and *env*) did not grow in macaque PBMC, whereas NM-3 carrying the 5′-genomic region of SIVmac (LTR, *gag*, *pol*, *vif* and *vpx*) did. Worthy of note, NM-8 containing *vif* and *vpr* of HIV-1 instead of *vif* and *vpx* of SIVmac did not replicate in macaque PBMC. These results indicated that the central region of the SIVmac genome (*vif* and/or *vpx*) is important for species tropism.

In addition to the determinant(s) mentioned above, the Gag-CA sequence has been suggested to be important for the tropism by analysing a chimeric virus designated SIV/HIV-CA [51]. This virus is an SIV derivative containing a portion of the HIV-1 *gag* gene that encodes the CA-p2 region, and was found to grow in human but not at all in macaque PBMC.

Overall, early studies on chimeric SIV/HIV viruses showed that viral determinants for species tropism exist in both the CA-p2 domain and the central genomic region of SIVmac.

TRIM5α

As mentioned above, HIV-1 infects human and chimpanzee cells but not cells of Old World monkeys [23,24]. HIV-1 is able to enter these monkey cells, but appears to be blocked before and/or during the reverse transcription process [24–26,52,53]. Since this restriction can be somewhat overcome by infection at a high dose or by pre-treating target cells with high titres of restricted virus-like particle [25,26,54,55], it was postulated that a saturable factor exists in restricting target cells.

A breakthrough in determining this saturable restriction factor came from the identification of TRIM5α. The significance of TRIM5α for HIV-1 restriction was demonstrated by the inhibition of HIV-1 infection in human cells expressing rhesus

Copyright © 2008 John Wiley & Sons, Ltd.

TRIM5α and by the rescue of HIV-1 infectivity in TRIM5α-knocked out rhesus cells by small interfering RNA [56]. Subsequent studies have revealed that the block of retrovirus replication with TRIM5α was species-specific. Human TRIM5α inhibited N-tropic murine leukaemia virus (N-MLV) and equine infectious anaemia virus (EIAV) replication [57,58], and TRIM5α from various Old World monkey species suppressed HIV-1 infection but not SIV [56,59]. TRIM proteins contain a tripartite motif defined by the presence of a RING (really interesting new gene) domain that possesses ubiquitin ligase acitivity, B-box and coiled-coil domain [60,61]. Coiled-coil domain is involved in multimerization of TRIM5α proteins [62,63]. TRIM5α encodes the C-terminal B30.2 (SPRY) domain that is absent in other TRIM5 isoforms. This SPRY domain is responsible for the direct interaction with viral CA and for the species-specific restriction of retroviral infection [64–68]. TRIM5α-mediated restriction can occur rapidly after virus entry into target cells and impede the reverse transcription process. The interaction of trimeric TRIM5α and multimerised viral CA leads to abnormally accelerated uncoating of incoming HIV-1 cores [69,70]. It has been suggested that either the reduction or increase of HIV-1 core stability with the mutations in CA impairs the reverse transcription [71] and that rapid disassembly of incoming HIV-1 cores mediated by TRIM5α results in restriction of HIV-1 replication [69,70].

Proteasome inhibitors can relieve rhesus TRIM5α-imposed block to the reverse transcription, and rescue the viral cDNA accumulation but not the productive infection of HIV-1 due to the inhibition of nuclear translocation of viral cDNA [72,73]. These observations suggest that TRIM5α may restrict another distinct step(s) in HIV-1 infection [73,74]. In any case, the exact mechanism by which TRIM5α blocks retroviral infection remains to be elucidated.

Cyclophilin A (CypA)

It was reported that HIV-1 CA binds to the cytoplasmic protein CypA [75]. Subsequent studies have indicated that CypA is efficiently incorporated into virions via interaction with HIV-1 CA domain in producer cells [76,77]. CypA also interacts with incoming HIV-1 cores in target cells and this interaction enhances HIV-1 infectivity [78,79]. In simian cells, reversely, CypA decreases HIV-1 infectivity depending on the presence of TRIM5α. Both cyclosporine A and a small interfering RNA, by blocking the interaction between HIV-1 CA/CypA and by decreasing CypA expression level, respectively, rescue HIV-1 infectivity through reducing HIV-1 sensitivity to TRIM5α in simian cells [80–83]. In contrast to the effect of CypA on TRIM5α-mediated restriction in simian cells, the interaction of CypA with the incoming HIV-1 core protects from TRIM5α restriction in human cells and CypA is required for maximal infectivity. It has been shown in human cells that the decrease of TRIM5α expression has little effect on HIV-1 infectivity and that the decrease of HIV-1 infectivity by blocking CA-CypA interaction is independent of TRIM5α expression, suggesting the existence of an unknown antiviral factor(s) in human cells [82–84]. Putative models for the recognition and interaction of CypA with TRIM5α and CA in human and simian cells have been proposed [74,85].

APOBEC3G/F

HIV-1 Vif has been shown to be essential for HIV-1 infectivity in certain cell types including primary lymphocytes, monocyte-derived macrophages and some T-cell lines [86–88]. After extensive efforts by many researchers, human A3G was finally identified as a cellular target of HIV-1 Vif [89]. A3G is a member of a polynucleotide cytidine deaminase family that displays diverse functions [90,91]. Since the discovery of A3G, it has been shown that other APOBEC family members also exhibit antiviral properties [92–95]. Of these, APOBEC3F (A3F) has a similarly strong antiviral activity to that of A3G and is countered by Vif [92,96–100].

In the absence of Vif, human A3G and A3F are incorporated into HIV-1 virions. On the infection of new target cells, the virion-associated A3G and A3F deaminate cytidine in viral minus-strand DNA during reverse transcription, leading to either the G to A hypermutation of viral genome or degradation of the viral genome by cellular DNA repair enzymes [101–105]. Since the cytidine deaminase-inactivated A3G and A3F still retain antiviral activity [100,106], it is quite clear that A3G and A3F have deaminase-independent antiviral activity [100,106–110].

Copyright © 2008 John Wiley & Sons, Ltd.

DOI: 10.1002/rmv

HIV-1 Vif degrades A3G and A3F via the ubiquitin–proteasome pathway by recruiting an E3 ubiquitin ligase complex with Cullin 5, Elongin B and Elongin C [99,111–114]. Some investigators have reported that Vif inhibits A3G antiviral activity via mechanisms independent of the ubiquitin–proteasome pathway [105,115–117]. The ability of Vif to induce the degradation of A3G is species-specific, and this specificity is probably determined by Vif/A3G binding. HIV-1 Vif interacts with and degrades human A3G amd A3F but not A3Gs derived from the rhesus macaque and African green monkey. In contrast, SIVmac Vif is able to inactivate both human and simian A3Gs [118]. Very recently, binding sites of human A3G and A3F in HIV-1 Vif have been identified. It was demonstrated that the distinct regions of Vif are required for interaction with A3G and A3F [119–121; manuscript in preparation].

GENERATION OF MONKEY CELL-TROPIC HIV-1 DERIVATIVES

Although SHIVs have been used for studies of the immune responses to primate immunodeficiency viruses [122,123], of the protective role of antibodies directed against the HIV-1 Env [35,124], and of the disease progression [49,50], the SHIV/monkey system itself has many serious scientific issues as described earlier. After the discovery of two major host cell restriction factors, TRIM5α and A3G (Figure 3), we initiated a research project to construct HIV-1/SIVmac chimeric viruses of a new and distinct category. We designed a hybrid virus in the backbone of the HIV-1 genome that can infect and grow in macaque monkey cells. In other words, we wished to make HIV-1 derivatives (genetically containing 90% or more sequences from HIV-1) having minimum essential sequences of SIVmac. Towards this end, the potentially important sequences within the HIV-1 genome against TRIM5α and A3G, that is *gag* and *vif* genes, were mutated or replaced with the corresponding regions of SIVmac.

In our laboratory, numbers of *gag*-chimeric viruses between HIV-1 and SIVmac239 already have been constructed. Some of them lost the infectivity even in human cells, and none of them showed the infectivity in cynomolgus monkey (CyM) HSC-F cells [125,126]. HSC-F is a CyM T-cell line originally immortalised by *Herpesvirus saimiri* [127]. Together with the data reported in early studies, these results prompted us to substitute both the CypA-binding loop in Gag-CA and the Vif with the corresponding sequences of SIVmac239 to generate monkey cell-tropic HIV-1 derivatives. As described below, we thus constructed an HIV-1 derivative designated pNL-ScaVR, which carries only a short seven-aa segment of *gag* gene corresponding to the HIV-1 CypA-binding loop and the entire *vif* gene from SIVmac [128]. The nine-aa CypA-binding loop in NL4-3 Gag-CA (HIV-1) was converted to the seven-residue MA239 CA analogue (SIVmac) by site-directed mutagenesis of a pNL4-3-derived *vif*-expression vector pNL-SX carrying the HIV-1 *vif* gene [129]. The full 214-aa Vif ORF from MA239 was amplified by PCR and inserted into this clone to generate a proviral construct pNL-ScaV. When expression of Gag, Pol, Env, Vpr, Vpu and Nef of pNL-ScaV was examined by transfection analysis, the level of Vpr was markedly reduced. Since this Vpr-defect was subsequently found to be caused by one of the *vif*-cloning sites in pNL-ScaV, a Vpr-repaired proviral clone designated pNL-ScaVR was constructed [128].

The SIVmac sequences in pNL-ScaVR were functionally active and counteracted effectively against the inhibitory factors in monkey cells as monitored by single-cycle replication assays for measuring *gag* and *vif* activities. As expected, the virus derived from pNL-ScaVR productively infected the CyM HSC-F cells. However, its growth kinetics were very much delayed relative to those of SIVmac from pMA239. Since it has been reported that HIV-1 acquires changes during extended tissue culture passage that confer augmented replicative properties [130,131], a viral adaptation experiment by long-term culture of infected HSC-F cells was performed. We successfully obtained viruses with enhanced growth abilities and finally have constructed by recombinant DNA techniques including PCR an infectious molecular clone (two biologically significant mutations in *env* gene) from them. It was designated pNL-DT5R [128] and used subsequently as a prototype clone (Figure 4). The NL-DT5R virus certainly grows in CD8-depleted PBMC prepared from pig-tailed and rhesus macaques and also in pig-tailed monkeys (see below), albeit less efficiently than SIVmac239. Another proviral clone designated stHIV-1, which contains the

Copyright © 2008 John Wiley & Sons, Ltd.

DOI: 10.1002/rmv

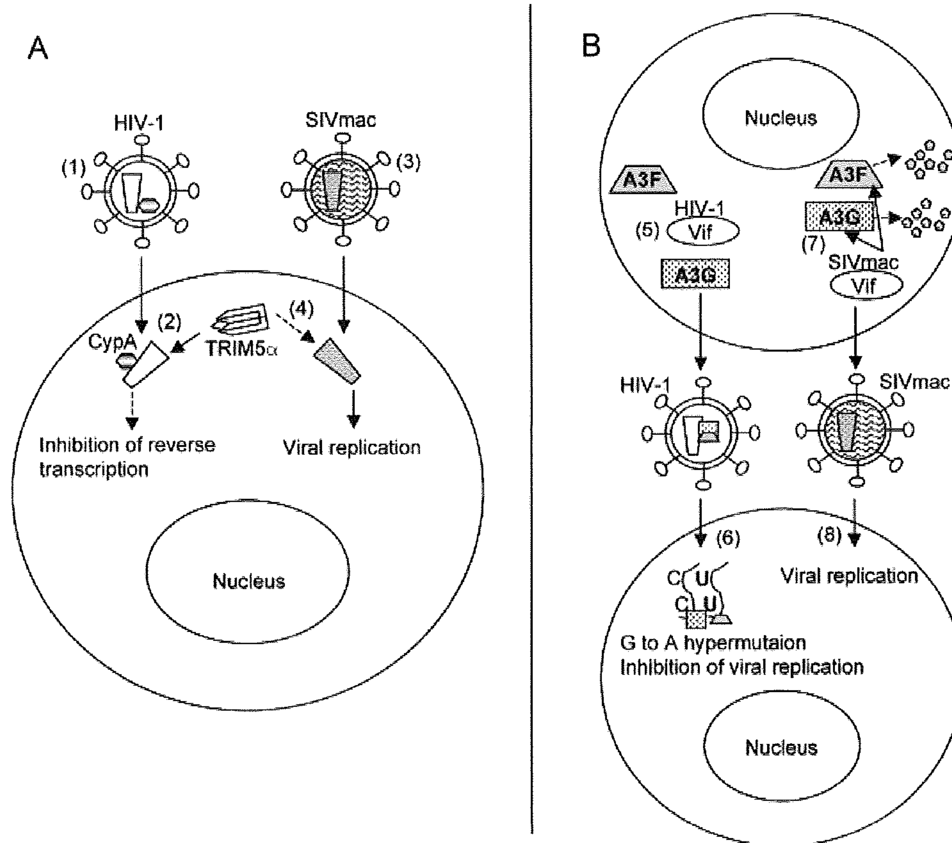


Figure 3. Schematic representation of species-specific restriction of HIV-1 replication by CypA/TRIM5α and by A3G/F in macaque monkey cells. Early HIV-1 replication steps affected by CypA/TRIM5α (A) and by A3G/F (B) are highlighted. (1) CypA is incorporated into HIV-1 virion. (2) After entry into cells, macaque TRIM5α interacts with CypA-CA core and promotes accelerated uncoating, resulting in the inhibition of reverse transcription. (3) No CypA is present in SIVmac virion. (4) In macaque cells, TRIM5α is unable to target SIVmac CA core and viral replication occurs efficiently. (5) HIV-1 Vif is unable to interact with and degrade macaque A3G/F, and both proteins are incorporated into HIV-1 virion. (6) In target cells, the virion-associated A3G/F deaminate cytidines in viral minus-strand DNA during reverse transcription, leading to the inhibition of viral replication by either G to A hypermutation in viral genome or the degradation of viral genome by cellular DNA repair enzyme. (7) SIVmac Vif inactivates macaque A3G/F in infected cells. (8) There is no carry-over of A3G/F, and SIVmac replication occurs normally in target cells

entire SIVmac CA and Vif coding sequences, was similarly constructed by others (Figure 4) and the virus was shown to be growth-competent in rhesus macaque PBMC [132]. Whether stHIV-1 can grow in monkeys is not yet reported.

To establish an ideal monkey model system for HIV-1/AIDS study, generation of HIV-1 derivatives that grow similarly well with SIVmac239, are pathogenic for macaque monkeys as well, and are R5-tropic, if possible, is essential. We already have started to modify the genome of NL-DT5R to further improve its growth potential in monkey cells. Extensive attempts to obtain *gag* and/or *vif* variants of NL-DT5R with an accelerated replication ability were unsuccessful (manuscripts in preparation; our unpublished data). Some of them were constructed based on the published reports [132,133]. Parallel attempts to construct R5-tropic viruses by *env*-substitution resulted in two full-length molecular clones designated pNL-DT5R5-1 and pNL-DT5R5-2 infectious for CD8-depleted PBMC of pig-tailed monkeys (our unpublished results). NL-DT5R5-1 was also infectious for HSC-F cells but less efficiently than the parental NL-DT5R as for PBMC. Clearly, biologically different from NL-DT5R, NL-DT5R5-1

Copyright © 2008 John Wiley & Sons, Ltd.

DOI: 10.1002/rmv

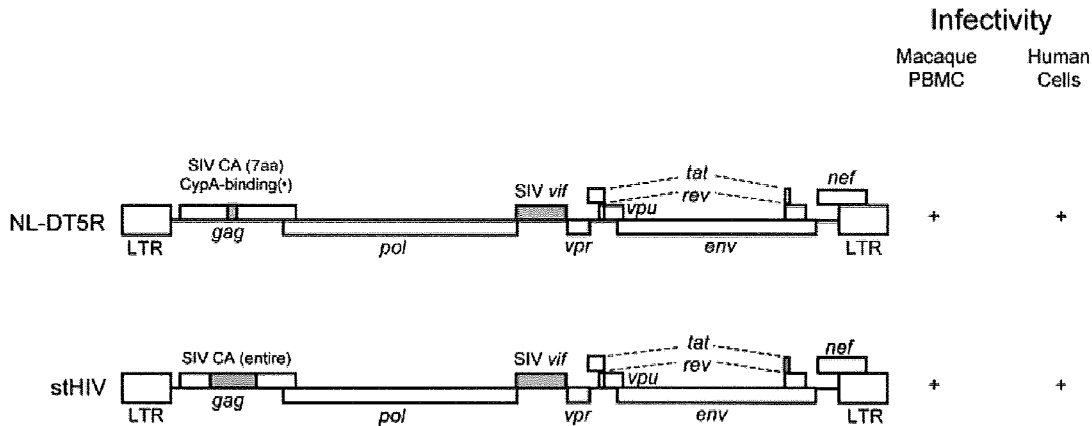


Figure 4. Genomic organisation and cellular tropism of HIV-1/SIVmac chimeric viruses of a novel class. White and grey boxes represent sequences from the parental clones NL4-3 (HIV-1) and MA239 (SIVmac), respectively. (+) on the right indicates growth-competence

rapidly induced severe cytopathic effects in HSC-F cells.

On the basis of our results and observations described above, we decided to modify the genomes of NL-DT5R and NL-DT5R5-1 by viral adaptation within HSC-F cells (Figure 5). Virus stocks prepared from transfected 293T cells were inoculated into HSC-F cells, and the cultures were maintained until progeny virus production was undetectable. Fresh HSC-F cells were then added to the cultures (on day 45 post-infection) and they were monitored for virus production. Viruses soon emerged in both co-cultures and grew to a higher level relative to that of viruses in early infection days. In order to ascertain the adapted nature of the emerged viruses, culture supernatants, collected from HSC-F cells infected with either NL-DT5R or NL-DT5R5-1 on day 57 (Figure 5) and normalised by RT activity, were inoculated into HSC-F cells (Figure 6). As is clear, the late

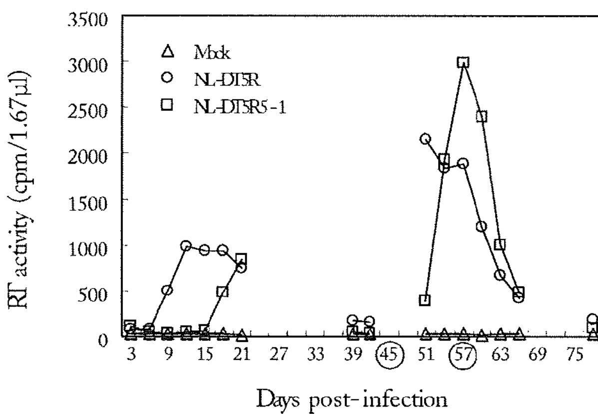


Figure 5. Growth properties of NL-DT5R (X4-tropic) and NL-DT5R5-1 (R5-tropic) viruses during a long-term passage in HSC-F cells. Virus samples were prepared from 293T cells transfected with the indicated proviral clones (Mock, pUC19), and equal RT units of viruses were inoculated into CyM HSC-F cells. Virus replication was monitored by RT production in the culture supernatants. On day 45 post-infection, fresh uninfected HSC-F cells were added to the cultures. On day 57 post-infection, cell-free viruses were prepared from the cultures, and used for monitoring their growth properties (see Figure 6)

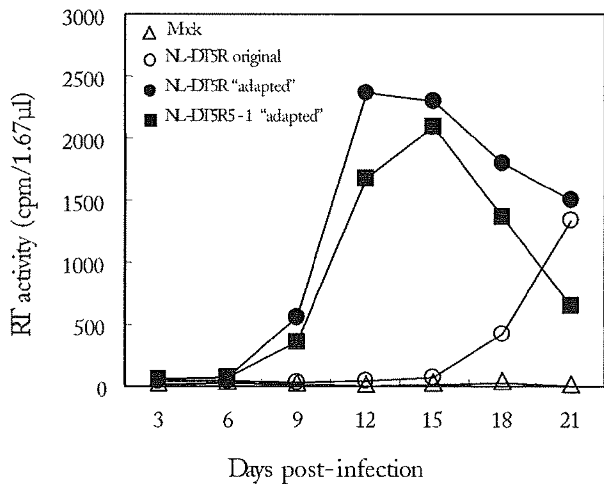


Figure 6. Growth kinetics of viruses generated during a long-term passage in HSC-F cells. Culture supernatants from NL-DT5R- or NL-DT5R5-1-infected HSC-F cells (adapted) were collected on day 57 post-infection (Figure 5), and equivalent RT units were inoculated into HSC-F cells. Virus replication was monitored by RT production in the culture supernatants. For controls, samples prepared from 293T cells transfected with NL-DT5R (original) or pUC19 (Mock) were used

Copyright © 2008 John Wiley & Sons, Ltd.

emerging viruses did in fact exhibit the adapted phenotype, that is, more robust viral replication and more extensive viral cytopathic effects than the original parental non-HSC-F-passage viruses. We have obtained a number of molecular clones from these adapted viruses by the method described above, and confirmed that viruses derived from them reproduce a similar biological property or even show a more enhanced phenotype in infected HSC-F cells (our unpublished results).

INFECTIONS OF MONKEYS WITH THE PROTOTYPE MONKEY CELL-TROPIC HIV-1 NL-DT5R

As a first monkey infection experiment of monkey cell-tropic HIV-1, NL-DT5R was inoculated into four pig-tailed macaques with/without anti-CD8 antibody treatment [134]. All the monkeys showed signs for viral productive and persistent infection. Plasma viral loads as monitored by viral RNA were elevated, reached a peak and gradually declined to an undetectable level by 5 to 11 weeks post-inoculation. Viral DNA in PBMC similarly persisted for 45 weeks post-infection. Virus in the infected animals could be transferred to another pig-tailed monkey. The NL-DT5R challenge into pig-tailed macaques also induced anti-HIV-1 antibody directed against HIV-1 encoded p17, p24, gp41, gp120 and gp160. However, the numbers of circulating CD4+ T-lymphocytes did not change appreciably during the observation period. Depletion of CD8+ cells had a small but significant effect on most of the above-described outcomes. In total, although NL-DT5R induced plasma viraemia and anti-HIV-1 antibodies in pig-tailed macaques, no significant depletion of CD4+ T-cells and no evidence of clinical disease were observed. Based on these results, other sets of monkey experiments are now taking off. Infection of CyMs, rhesus monkeys and pig-tailed monkeys by NL-DT5R and its improved versions may be a milestone for the establishment of monkey models for HIV-1/AIDS study.

CONCLUSIONS AND FUTURE DIRECTIONS

Recently discovered and extensively studied host cell restriction factors against HIV-1 have given us a rationale for generation of monkey-tropic HIV-1 to establish a pivotal monkey system for both basic and clinical studies. These factors have explained, at least in part, the narrow host range of HIV-1, and a number of new HIV-1s that are able to infect monkey cells have been generated by us and others [128,132; our unpublished data]. The genome of monkey-tropic HIV-1s basically contains both a 21-nucleotide SIVmac239 Gag-CA element, corresponding to the HIV-1 CypA-binding site, and the entire SIVmac 239 *vif* gene (Figure 4). While the prototype monkey-tropic HIV-1 designated NL-DT5R clearly established spreading infections in a CyM T-cell line, CD8-depleted PBMCs from pig-tailed and rhesus macaques, it did not cause AIDS-like symptoms at all in pig-tailed monkeys. Because the virus grows more poorly than the SIVmac239 virus both in cultured simian cells and in monkeys, improvement of viral replication by the modification of the viral genome is required for developing tractable monkey models for HIV-1/AIDS study. Our extensive attempts to obtain such variant viruses through genetic manipulation of *gag* and *vif* genes have so far been unsuccessful. In sharp contrast, viral adaptation in cells to speed up the growth rate appeared to occur quite efficiently (Figures 5 and 6), and infectious molecular clones with adapted viral phenotypes tropic either for X4 or R5 cells were obtained (our unpublished results). Sequencing the genome of adapted viruses and subsequent functional analysis may reveal the presence of an otherwise undiscovered genetic region(s) responsible for the species tropism of HIV-1. We have been convinced that the above-mentioned new viral clones would give us a good chance to develop HIV-1/AIDS-nonhuman primate models. According to recent reports [135,136], incorporation of green fluorescent protein (GFP) into HIV-1 clones enables one to visualise the virus itself. We are interested in generating 'visible viruses', since they are eminently useful to determine the movement of viruses in monkeys that would be involved in or associated with disease progression.

Needless to say, monkey infection models provide powerful tools for the elucidation of the unknown role of HIV-1 accessory proteins in the HIV-1 life cycle and pathogenesis, and more importantly, for the development of vaccines and drugs for the prevention and treatment. In a different point of view, we are also interested in analysing, by determining the alterations of viral genomes, the difference of responses against HIV-1 infection among individuals and/or

Copyright © 2008 John Wiley & Sons, Ltd.

DOI: 10.1002/rmv

species. HIV-1 may variably mutate and evolve during the course of spreading persistent infection. The information from these studies would offer new avenues for clinical features as well as basic research in HIV-1.

REFERENCES

1. De Clercq E. HIV inhibitors targeted at the reverse transcriptase. *AIDS Res Hum Retroviruses* 1992; **8**(2): 119–134.
2. Uberla K, Stahl-Henning C, Bottiger D, *et al.* Animal model for the therapy of acquired immunodeficiency syndrome with reverse transcriptase inhibitors. *Proc Natl Acad Sci USA* 1995; **92**(18): 8210–8214.
3. Witvrouw M, Pannecouque C, Switzer WM, *et al.* Susceptibility of HIV-2, SIV and SHIV to various anti-HIV-1 compounds: implications for treatment and postexposure prophylaxis. *Antivir Ther* 2004; **9**(1): 57–65.
4. Nishimura Y, Igarashi T, Donau OK, *et al.* Highly pathogenic SHIVs and SIVs target different CD4+ T cell subsets in rhesus monkeys, explaining their divergent clinical courses. *Proc Natl Acad Sci USA* 2004; **101**(33): 12324–12329.
5. Nishimura Y, Brown CR, Mattapallil JJ, *et al.* Resting naive CD4+ T cells are massively infected and eliminated by X4-tropic simian-human immunodeficiency viruses in macaques. *Proc Natl Acad Sci USA* 2005; **102**(22): 8000–8005.
6. Brown CR, Czapiga M, Kabat J, *et al.* Unique pathology in simian immunodeficiency virus-infected rapid progressor macaques is consistent with a pathogenesis distinct from that of classical AIDS. *J Virol* 2007; **81**(11): 5594–5606.
7. Kanki PJ, McLane MF, King NW Jr, *et al.* Serologic identification and characterization of a macaque T-lymphotropic retrovirus closely related to HTLV-III. *Science* 1985; **228**(4704): 1199–1201.
8. Daniel MD, Letvin NL, King NW, *et al.* Isolation of T-cell tropic HTLV-III-like retrovirus from macaques. *Science* 1985; **228**(4704): 1201–1204.
9. Letvin NL, Daniel MD, Sehgal PK, *et al.* Induction of AIDS-like disease in macaque monkeys with T-cell tropic retrovirus STLV-III. *Science* 1985; **230**(4721): 71–73.
10. Naidu YM, Kestler HW III, Li Y, *et al.* Characterization of infectious molecular clones of simian immunodeficiency virus (SIVmac) and human immunodeficiency virus type 2: persistent infection of rhesus monkeys with molecularly cloned SIVmac. *J Virol* 1988; **62**(12): 4691–4696.
11. Kestler H, Kodama T, Ringler D, *et al.* Induction of AIDS in rhesus monkeys by molecularly cloned simian immunodeficiency virus. *Science* 1990; **248**(4959): 1109–1112.
12. Murphey-Corb M, Martin LN, Rangan SR, *et al.* Isolation of an HTLV-III-related retrovirus from macaques with simian AIDS and its possible origin in asymptomatic mangabeys. *Nature* 1986; **321**(6068): 435–437.
13. Fultz PN, McClure HM, Anderson DC, *et al.* Isolation of a T-lymphotropic retrovirus from naturally infected sooty mangabey monkeys (Cercocebus atys). *Proc Natl Acad Sci USA* 1986; **83**(14): 5286–5290.
14. Hirsch VM, Olmsted RA, Murphey-Corb M, *et al.* An African primate lentivirus (SIVsm) closely related to HIV-2. *Nature* 1989; **339**(6223): 389–392.
15. Fukasawa M, Miura T, Hasegawa A, *et al.* Sequence of simian immunodeficiency virus from African green monkey, a new member of the HIV/SIV group. *Nature* 1988; **333**(6172): 457–461.
16. Daniel MD, Li Y, Naidu YM, *et al.* Simian immunodeficiency virus from African green monkeys. *J Virol* 1988; **62**(11): 4123–4128.
17. Allan JS, Short M, Taylor ME, *et al.* Species-specific diversity among simian immunodeficiency viruses from African green monkeys. *J Virol* 1991; **65**(6): 2816–2818.
18. Tsujimoto H, Hasegawa A, Maki N, *et al.* Sequence of a novel simian immunodeficiency virus from a wild-caught African mandrill. *Nature* 1989; **341**(6242): 539–541.
19. Souquiere S, Bibollet-Ruche F, Robertson DL, *et al.* Wild Mandrillus sphinx are carriers of two types of lentivirus. *J Virol* 2001; **75**(15): 7086–7096.
20. Hirsch VM, Dapolite G, Johnson PR, *et al.* Induction of AIDS by simian immunodeficiency virus from an African green monkey: species-specific variation in pathogenicity correlates with the extent of in vivo replication. *J Virol* 1995; **69**(2): 955–967.
21. Desrosiers RC. The simian immunodeficiency viruses. *Annu Rev Immunol* 1990; **8**: 557–578.
22. Hirsch VM, Johnson PR. Pathogenic diversity of simian immunodeficiency viruses. *Virus Res* 1994; **32**(2): 183–203.
23. Shibata R, Kawamura M, Sakai H, *et al.* Generation of a chimeric human and simian immunodeficiency virus infectious to monkey peripheral blood mononuclear cells. *J Virol* 1991; **65**(7): 3514–3520.
24. Hofmann W, Schubert D, LaBonte J, *et al.* Species-specific, postentry barriers to primate immunodeficiency virus infection. *J Virol* 1999; **73**(12): 10020–10028.
25. Cowan S, Hatziioannou T, Cunningham T, *et al.* Cellular inhibitors with Fv1-like activity restrict human and simian immunodeficiency virus tropism. *Proc Natl Acad Sci USA* 2002; **99**(18): 11914–11919.

Copyright © 2008 John Wiley & Sons, Ltd.

DOI: 10.1002/rmv

26. Besnier C, Takeuchi Y, Towers G. Restriction of lentivirus in monkeys. *Proc Natl Acad Sci USA* 2002; **99**(18): 11920–11925.
27. Shibata R, Adachi A. SIV/HIV recombinants and their use in studying biological properties. *AIDS Res Hum Retroviruses* 1992; **8**(3): 403–409.
28. Luciw PA, Pratt-Lowe E, Shaw KE, *et al.* Persistent infection of rhesus macaques with T-cell-line-tropic and macrophage-tropic clones of simian/human immunodeficiency viruses (SHIV). *Proc Natl Acad Sci USA* 1995; **92**(16): 7490–7494.
29. Joag SV, Li Z, Foresman L, *et al.* Chimeric simian/human immunodeficiency virus that causes progressive loss of CD4+ T cells and AIDS in pig-tailed macaques. *J Virol* 1996; **70**(5): 3189–3197.
30. Reimann KA, Li JT, Veazey R, *et al.* A chimeric simian/human immunodeficiency virus expressing a primary patient human immunodeficiency virus type 1 isolate env causes an AIDS-like disease after in vivo passage in rhesus monkeys. *J Virol* 1996; **70**(10): 6922–6928.
31. Igarashi T, Endo Y, Englund G, *et al.* Emergence of a highly pathogenic simian/human immunodeficiency virus in a rhesus macaque treated with anti-CD8 mAb during a primary infection with a nonpathogenic virus. *Proc Natl Acad Sci USA* 1999; **96**(24): 14049–14054.
32. Shibata R, Igarashi T, Haigwood N, *et al.* Neutralizing antibody directed against the HIV-1 envelope glycoprotein can completely block HIV-1/SIV chimeric virus infections of macaque monkeys. *Nat Med* 1999; **5**(2): 204–210.
33. Nishimura Y, Igarashi T, Haigwood NL, *et al.* Transfer of neutralizing IgG to macaques 6 h but not 24 h after SHIV infection confers sterilizing protection: implications for HIV-1 vaccine development. *Proc Natl Acad Sci USA* 2003; **100**(25): 15131–15136.
34. Ambrose Z, Boltz V, Palmer S, *et al.* In vitro characterization of a simian immunodeficiency virus-human immunodeficiency virus (HIV) chimera expressing HIV type 1 reverse transcriptase to study antiviral resistance in pigtail macaques. *J Virol* 2004; **78**(24): 13553–13561.
35. Amara R, Villinger F, Altman JD, *et al.* Control of a mucosal challenge and prevention of AIDS by a multiprotein DNA/MVA vaccine. *Science* 2001; **292**(5514): 69–74.
36. Matano T, Kano M, Nakamura H, *et al.* Rapid appearance of secondary immune responses and protection from acute CD4 depletion after a highly pathogenic immunodeficiency virus challenge in macaques vaccinated with a DNA prime/Sendai virus vector boost regimen. *J Virol* 2001; **75**(23): 11891–11896.
37. Shiver JW, Fu TM, Chen L, *et al.* Replication-incompetent adenoviral vaccine vector elicits effective anti-immunodeficiency-virus immunity. *Nature* 2002; **415**(6869): 331–335.
38. Casimiro DR, Wang F, Schleif WA, *et al.* Attenuation of simian immunodeficiency virus SIVmac239 infection by prophylactic immunization with dna and recombinant adenoviral vaccine vectors expressing Gag. *J Virol* 2005; **79**(24): 15547–15555.
39. Mattapallil JJ, Douek DC, Buckler-White A, *et al.* Vaccination preserves CD4 memory T cells during acute simian immunodeficiency virus challenge. *J Exp Med* 2006; **203**(6): 1533–1541.
40. Letvin NL, Mascola JR, Sun Y, *et al.* Preserved CD4+ central memory T cells and survival in vaccinated SIV-challenged monkeys. *Science* 2006; **312**(5779): 1530–1533.
41. Veazey RS, DeMaria M, Chalifoux LV, *et al.* Gastrointestinal tract as a major site of CD4+ T cell depletion and viral replication in SIV infection. *Science* 1998; **280**(5362): 427–431.
42. Mattapallil JJ, Douek DC, Hill B, *et al.* Massive infection and loss of memory CD4+ T cells in multiple tissues during acute SIV infection. *Nature* 2005; **434**(7037): 1093–1097.
43. Li Q, Duan L, Estes JD, *et al.* Peak SIV replication in resting memory CD4+ T cells depletes gut lamina propria CD4+ T cells. *Nature* 2005; **434**(7037): 1148–1152.
44. Harouse JM, Gettie A, Tan RC, *et al.* Distinct pathogenic sequela in rhesus macaques infected with CCR5 or CXCR4 utilizing SHIVs. *Science* 1999; **284**(5415): 816–819.
45. Tan RC, Harouse JM, Gettie A, *et al.* In vivo adaptation of SHIV(SF162): chimeric virus expressing a NSI, CCR5-specific envelope protein. *J Med Primatol* 1999; **28**(4-5): 164–168.
46. Song RJ, Chenine AL, Rasmussen RA, *et al.* Molecularly cloned SHIV-1157ipd3N4: a highly replication- competent, mucosally transmissible R5 simian-human immunodeficiency virus encoding HIV clade C Env. *J Virol* 2006; **80**(17): 8729–8738.
47. Misumi S, Nakayama D, Kusaba M, *et al.* Effects of immunization with CCR5-based cycloimmunogen on simian/HIVSF162P3 challenge. *J Immunol* 2006; **176**(1): 463–471.
48. Cristillo AD, Lisziewicz J, He L, *et al.* HIV-1 prophylactic vaccine comprised of topical DermaVir prime and protein boost elicits cellular immune responses and controls pathogenic R5 SHIV162P3. *Virology* 2007; **366**(1): 197–211.
49. Ho SH, Shek L, Gettie A, *et al.* V3 loop-determined coreceptor preference dictates the dynamics

Copyright © 2008 John Wiley & Sons, Ltd.

of CD4+-T-cell loss in simian-human immunodeficiency virus-infected macaques. *J Virol* 2005; **79**(19): 12296–12303.
50. Tsai L, Trunova N, Gettie A, *et al.* Efficient repeated low-dose intravaginal infection with X4 and R5 SHIVs in rhesus macaque: implications for HIV-1 transmission in humans. *Virology* 2007; **362**(1): 207–216.
51. Dorfman T, Göttlinger HG. The human immunodeficiency virus type 1 capsid p2 domain confers sensitivity to the cyclophilin-binding drug SDZ NIM 811. *J Virol* 1996; **70**(9): 5751–5757.
52. Shibata R, Sakai H, Kawamura M, *et al.* Early replication block of human immunodeficiency virus type 1 in monkey cells. *J Gen Virol* 1995; **76**(Pt 11): 2723–2730.
53. Towers G, Bock M, Matin S, *et al.* A conserved mechanism of retrovirus restriction in mammals. *Proc Natl Acad Sci USA* 2000; **97**(22): 12295–12299.
54. Towers G, Collins M, Takeuchi Y. Abrogation of Ref1 retrovirus restriction in human cells. *J Virol* 2002; **76**(5): 2548–2550.
55. Hatziioannou T, Cowan S, Goff SP, *et al.* Restriction of multiple divergent retroviruses by Lv1 and Ref1. *EMBO J* 2003; **22**(3): 385–394.
56. Stremlau M, Owens CM, Perron MJ, *et al.* The cytoplasmic body component TRIM5alpha restricts HIV-1 infection in Old World monkeys. *Nature* 2004; **427**(6977): 848–853.
57. Yap MW, Nisole S, Lynch C, *et al.* Trim5alpha protein restricts both HIV-1 and murine leukemia virus. *Proc Natl Acad Sci USA* 2004; **101**(29): 10786–10791.
58. Perron MJ, Stremlau M, Song B, *et al.* TRIM5alpha mediates the postentry block to N-tropic murine leukemia viruses in human cells. *Proc Natl Acad Sci USA* 2004; **101**(32): 11827–11832.
59. Song B, Javanbakht H, Perron M, *et al.* Retrovirus restriction by TRIM5alpha variants from Old World and New World primates. *J Virol* 2005; **79**(7): 3930–3937.
60. Borden KL, Lally JM, Martin SR, *et al.* Novel topology of a zinc-binding domain from a protein involved in regulating early Xenopus development. *EMBO J* 1995; **14**(23): 5947–5956.
61. Reymond A, Meroni G, Fantozzi A, *et al.* The tripartite motif family identifies cell compartments. *EMBO J* 2001; **20**(9): 2140–2151.
62. Massiah MA, Simmons BN, Short KM, *et al.* Solution structure of the RBCC/TRIM B-box1 domain of human MID1: B-box with a RING. *J Mol Biol* 2006; **358**(2): 532–545.
63. Massiah MA, Matts JA, Short KM, *et al.* Solution structure of the MID1 B-box2 CHC(D/C)C(2)H(2) zinc-binding domain: insights into an evolutionarily conserved RING fold. *J Mol Biol* 2007; **369**(1): 1–10.
64. Yap MW, Nisole S, Stoye JP. A single amino acid change in the SPRY domain of human Trim5alpha leads to HIV-1 restriction. *Curr Biol* 2005; **15**(1): 73–78.
65. Stremlau M, Perron M, Welikala S, *et al.* Species-specific variation in the B30.2(SPRY) domain of TRIM5alpha determines the potency of human immunodeficiency virus restriction. *J Virol* 2005; **79**(5): 3139–3145.
66. Nakayama EE, Miyoshi H, Nagai Y, *et al.* A specific region of 37 amino acid residues in the SPRY (B30.2) domain of African green monkey TRIM5-alpha determines species-specific restriction of simian immunodeficiency virus SIVmac infection. *J Virol* 2005; **79**(14): 8870–8877.
67. Perez-Caballero D, Hatziioannou T, Yang A, *et al.* Human tripartite motif 5alpha domains responsible for retrovirus restriction activity and specificity. *J Virol* 2005; **79**(14): 8969–8978.
68. Perron MJ, Stremlau M, Sodroski J. Two surface-exposed elements of the B30.2/SPRY domain as potency determinants of N-tropic murine leukemia virus restriction by human TRIM5alpha. *J Virol* 2006; **80**(11): 5631–5636.
69. Stremlau M, Perron M, Lee M, *et al.* Specific recognition and accelerated uncoating of retroviral capsids by the TRIM5alpha restriction factor. *Proc Natl Acad Sci USA* 2006; **103**(14): 5514–5519.
70. Perron MJ, Stremlau M, Lee M, *et al.* The human TRIM5alpha restriction factor mediates accelerated uncoating of the N-tropic murine leukemia virus capsid. *J Virol* 2007; **81**(5): 2138–2148.
71. Forshey BM, von Schwedler U, Sundquist WI, *et al.* Formation of a human immunodeficiency virus type 1 core of optimal stability is crucial for viral replication. *J Virol* 2002; **76**(11): 5667–5677.
72. Wu X, Anderson JL, Campbell EM, *et al.* Proteasome inhibitors uncouple rhesus TRIM5alpha restriction of HIV-1 reverse transcription and infection. *Proc Natl Acad Sci USA* 2006; **103**(19): 7465–7470.
73. Anderson JL, Campbell EM, Wu X, *et al.* Proteasome inhibition reveals that a functional preintegration complex intermediate can be generated during restriction by diverse TRIM5 proteins. *J Virol* 2006; **80**(19): 9754–9760.
74. Luban J. Cyclophilin A, TRIM5, and resistance to human immunodeficiency virus type 1 infection. *J Virol* 2007; **81**(3): 1054–1061.
75. Luban J, Bossolt KL, Franke EK, *et al.* Human immunodeficiency virus type 1 Gag protein binds to cyclophilins A and B. *Cell* 1993; **73**(6): 1067–1078.

Copyright © 2008 John Wiley & Sons, Ltd.

DOI: 10.1002/rmv

76. Franke EK, Yuan HE, Luban J. Specific incorporation of cyclophilin A into HIV-1 virions. *Nature* 1994; **372**(6504): 359–362.
77. Thali M, Bukovsky A, Kondo E, *et al.* Functional association of cyclophilin A with HIV-1 virions. *Nature* 1994; **372**(6504): 363–365.
78. Hatziioannou T, Perez-Caballero D, Cowan S, *et al.* Cyclophilin interactions with incoming human immunodeficiency virus type 1 capsids with opposing effects on infectivity in human cells. *J Virol* 2005; **79**(1): 176–183.
79. Sokolskaja E, Sayah DM, Luban J. Target cell cyclophilin A modulates human immunodeficiency virus type 1 infectivity. *J Virol* 2004; **78**(23): 12800–12808.
80. Sayah DM, Sokolskaja E, Berthoux L, *et al.* Cyclophilin A retrotransposition into TRIM5 explains owl monkey resistance to HIV-1. *Nature* 2004; **430**(6999): 569–573.
81. Berthoux L, Sebastian S, Sokolskaja E, *et al.* Cyclophilin A is required for TRIM5{alpha}-mediated resistance to HIV-1 in Old World monkey cells. *Proc Natl Acad Sci USA* 2005; **102**(41): 14849–14853.
82. Keckesova Z, Ylinen L, Towers GJ. Cyclophilin A renders human immunodeficiency virus type 1 sensitive to Old World monkey but not human TRIM5 alpha antiviral activity. *J Virol* 2006; **80**(10): 4683–4690.
83. Stremlau M, Song B, Javanbakht H, *et al.* Cyclophilin A: an auxiliary but not necessary cofactor for TRIM5alpha restriction of HIV-1. *Virology* 2006; **351**(1): 112–120.
84. Sokolskaja E, Berthoux L, Luban J. Cyclophilin A and TRIM5alpha independently regulate human immunodeficiency virus type 1 infectivity in human cells. *J Virol* 2006; **80**(6): 2855–2862.
85. Towers GJ. The control of viral infection by tripartite motif proteins and cyclophilin A. *Retrovirology* 2007; **4**: 40.
86. Sodroski J, Goh WC, Rosen C, *et al.* Replicative and cytopathic potential of HTLV-III/LAV with sor gene deletions. *Science* 1986; **231**(4745): 1549–1553.
87. Strebel K, Daugherty D, Clouse K, *et al.* The HIV 'A' (sor) gene product is essential for virus infectivity. *Nature* 1987; **328**(6132): 728–730.
88. Fisher AG, Ensoli B, Ivanoff L, *et al.* The sor gene of HIV-1 is required for efficient virus transmission in vitro. *Science* 1987; **237**(4817): 888–893.
89. Sheehy AM, Gaddis NC, Choi JD, *et al.* Isolation of a human gene that inhibits HIV-1 infection and is suppressed by the viral Vif protein. *Nature* 2002; **418**(6898): 646–650.
90. Harris RS, Liddament MT. Retroviral restriction by APOBEC proteins. *Nat Rev Immunol* 2004; **4**(11): 868–877.
91. Holmes RK, Malim MH, Bishop KN. APOBEC-mediated viral restriction: not simply editing? *Trends Biochem Sci* 2007; **32**(3): 118–128.
92. Bishop KN, Holmes RK, Sheehy AM, *et al.* Cytidine deamination of retroviral DNA by diverse APOBEC proteins. *Curr Biol* 2004; **14**(15): 1392–1396.
93. Doehle BP, Schäfer A, Cullen BR. Human APOBEC3B is a potent inhibitor of HIV-1 infectivity and is resistant to HIV-1 Vif. *Virology* 2005; **339**(2): 281–288.
94. Rose KM, Marin M, Kozak SL, *et al.* Regulated production and anti-HIV type 1 activities of cytidine deaminases APOBEC3B, 3F, and 3G. *AIDS Res Hum Retroviruses* 2005; **21**(7): 611–619.
95. Dang Y, Wang X, Esselman WJ, *et al.* Identification of APOBEC3DE as another antiretroviral factor from the human APOBEC family. *J Virol* 2006; **80**(21): 10522–10533.
96. Liddament MT, Brown WL, Schumacher AJ, *et al.* APOBEC3F properties and hypermutation preferences indicate activity against HIV-1 in vivo. *Curr Biol* 2004; **14**(15): 1385–1391.
97. Wiegand HL, Doehle BP, Bogerd HP, *et al.* A second human antiretroviral factor, APOBEC3F, is suppressed by the HIV-1 and HIV-2 Vif proteins. *EMBO J* 2004; **23**(12): 2451–2458.
98. Zheng YH, Irwin D, Kurosu T, *et al.* Human APOBEC3F is another host factor that blocks human immunodeficiency virus type 1 replication. *J Virol* 2004; **78**(11): 6073–6076.
99. Liu B, Sarkis PT, Luo K, *et al.* Regulation of Apobec3F and human immunodeficiency virus type 1 Vif by Vif-Cul5-ElonB/C E3 ubiquitin ligase. *J Virol* 2005; **79**(15): 9579–9587.
100. Holmes RK, Koning FA, Bishop KN, *et al.* APOBEC3F can inhibit the accumulation of HIV-1 reverse transcription products in the absence of hypermutation. Comparisons with APOBEC3G. *J Biol Chem* 2007; **282**(4): 2587–2595.
101. Lecossier D, Bouchonnet F, Clavel F, *et al.* Hypermutation of HIV-1 DNA in the absence of the Vif protein. *Science* 2003; **300**(5622): 1112.
102. Harris RS, Bishop KN, Sheehy AM, *et al.* DNA deamination mediates innate immunity to retroviral infection. *Cell* 2003; **113**(6): 803–809. Erratum in: *Cell* 2004; **116**(4): 629.
103. Zhang H, Yang B, Pomerantz RJ, *et al.* The cytidine deaminase CEM15 induces hypermutation in newly synthesized HIV-1 DNA. *Nature* 2003; **424**(6944): 94–98.
104. Mangeat B, Turelli P, Caron G, *et al.* Broad antiretroviral defence by human APOBEC3G through lethal editing of nascent reverse transcripts. *Nature* 2003; **424**(6944): 99–103.

Copyright © 2008 John Wiley & Sons, Ltd.

105. Mariani R, Chen D, Schröfelbauer B, *et al.* Species-specific exclusion of APOBEC3G from HIV-1 virions by Vif. *Cell* 2003; **114**(1): 21–31.
106. Newman EN, Holmes RK, Craig HM, *et al.* Antiviral function of APOBEC3G can be dissociated from cytidine deaminase activity. *Curr Biol* 2005; **15**(2): 166–170.
107. Shindo K, Takaori-Kondo A, Kobayashi M, *et al.* The enzymatic activity of CEM15/Apobec-3G is essential for the regulation of the infectivity of HIV-1 virion but not a sole determinant of its antiviral activity. *J Biol Chem* 2003; **278**(45): 44412–44416.
108. Chiu YL, Soros VB, Kreisberg JF, *et al.* Cellular APOBEC3G restricts HIV-1 infection in resting CD4+ T cells. *Nature* 2005; **435**(7038): 108–114.
109. Bishop KN, Holmes RK, Malim MH. Antiviral potency of APOBEC proteins does not correlate with cytidine deamination. *J Virol* 2006; **80**(17): 8450–8458.
110. Iwatani Y, Chan DS, Wang F, *et al.* Deaminase-independent inhibition of HIV-1 reverse transcription by APOBEC3G. *Nucleic Acids Res* 2007; **35**(21): 7096–7108.
111. Marin M, Rose KM, Kozak SL, *et al.* HIV-1 Vif protein binds the editing enzyme APOBEC3G and induces its degradation. *Nat Med* 2003; **9**(11): 1398–1403.
112. Sheehy AM, Gaddis NC, Malim MH. The antiretroviral enzyme APOBEC3G is degraded by the proteasome in response to HIV-1 Vif. *Nat Med* 2003; **9**(11): 1404–1407.
113. Yu X, Yu Y, Liu B, *et al.* Induction of APOBEC3G ubiquitination and degradation by an HIV-1 Vif-Cul5-SCF complex. *Nature* 2003; **302**(5647): 1056–1060.
114. Mehle A, Strack B, Ancuta P, *et al.* Vif overcomes the innate antiviral activity of APOBEC3G by promoting its degradation in the ubiquitin-proteasome pathway. *J Biol Chem* 2004; **279**(9): 7792–7798.
115. Stopak K, de Noronha C, Yonemoto W, *et al.* HIV-1 Vif blocks the antiviral activity of APOBEC3G by impairing both its translation and intracellular stability. *Mol Cell* 2003; **12**(3): 591–601.
116. Kao S, Khan MA, Miyagi E, *et al.* The human immunodeficiency virus type 1 Vif protein reduces intracellular expression and inhibits packaging of APOBEC3G (CEM15), a cellular inhibitor of virus infectivity. *J Virol* 2003; **77**(21): 11398–11407.
117. Santa-Marta M, da Silva FA, Fonseca AM, *et al.* HIV-1 Vif can directly inhibit apolipoprotein B mRNA-editing enzyme catalytic polypeptide-like 3G-mediated cytidine deamination by using a single amino acid interaction and without protein degradation. *J Biol Chem* 2005; **280**(10): 8765–8775.
118. Cullen BR. Role and mechanism of action of the APOBEC3 family of antiretroviral resistance factors. *J Virol* 2006; **80**(3): 1067–1076.
119. Tian C, Yu X, Zhang W, *et al.* Differential requirement for conserved tryptophans in human immunodeficiency virus type 1 Vif for the selective suppression of APOBEC3G and APOBEC3F. *J Virol* 2006; **80**(6): 3112–3115.
120. Russell RA, Pathak VK. Identification of two distinct human immunodeficiency virus type 1 Vif determinants critical for interactions with human APOBEC3G and APOBEC3F. *J Virol* 2007; **81**(15): 8201–8210.
121. Mehle A, Wilson H, Zhang C, *et al.* Identification of an APOBEC3G binding site in human immunodeficiency virus type 1 Vif and inhibitors of Vif-APOBEC3G binding. *J Virol* 2007; **81**(23): 13235–13241.
122. Chen ZW, Kou ZC, Lekutis C, *et al.* T cell receptor V beta repertoire in an acute infection of rhesus monkeys with simian immunodeficiency viruses and a chimeric simian-human immunodeficiency virus. *J Exp Med* 1995; **182**(1): 21–31.
123. Mascola JR, Lewis MG, VanCott TC, *et al.* Cellular immunity elicited by human immunodeficiency virus type 1/simian immunodeficiency virus DNA vaccination does not augment the sterile protection afforded by passive infusion of neutralizing antibodies. *J Virol* 2003; **77**(19): 10348–10356.
124. Nishimura Y, Igarashi T, Haigwood N, *et al.* Determination of a statistically valid neutralization titer in plasma that confers protection against simian-human immunodeficiency virus challenge following passive transfer of high-titered neutralizing antibodies. *J Virol* 2002; **76**(5): 2123–2130.
125. Fujita M, Yoshida A, Miyaura M, *et al.* Cyclophilin A-independent replication of a human immunodeficiency virus type 1 isolate carrying a small portion of the simian immunodeficiency virus SIV(MAC) gag capsid region. *J Virol* 2001; **75**(21): 10527–10531.
126. Kamada K, Yoshida A, Khamsri B, *et al.* Construction of gag-chimeric viruses between HIV-1 and SIVmac that are capable of productive multi-cycle infection. *Microbes Infect* 2006; **8**(4): 1075–1081.
127. Akari H, Fukumori T, Iida S, *et al.* Induction of apoptosis in *Herpesvirus saimiri*-immortalized T lymphocytes by blocking interaction of CD28 with CD80/CD86. *Biochem Biophys Res Commun* 1999; **263**(2): 352–356.
128. Kamada K, Igarashi T, Martin MA, *et al.* Generation of HIV-1 derivatives that productively infect macaque monkey lymphoid cells. *Proc Natl Acad Sci USA* 2006; **103**(45): 16959–16964.

Copyright © 2008 John Wiley & Sons, Ltd.