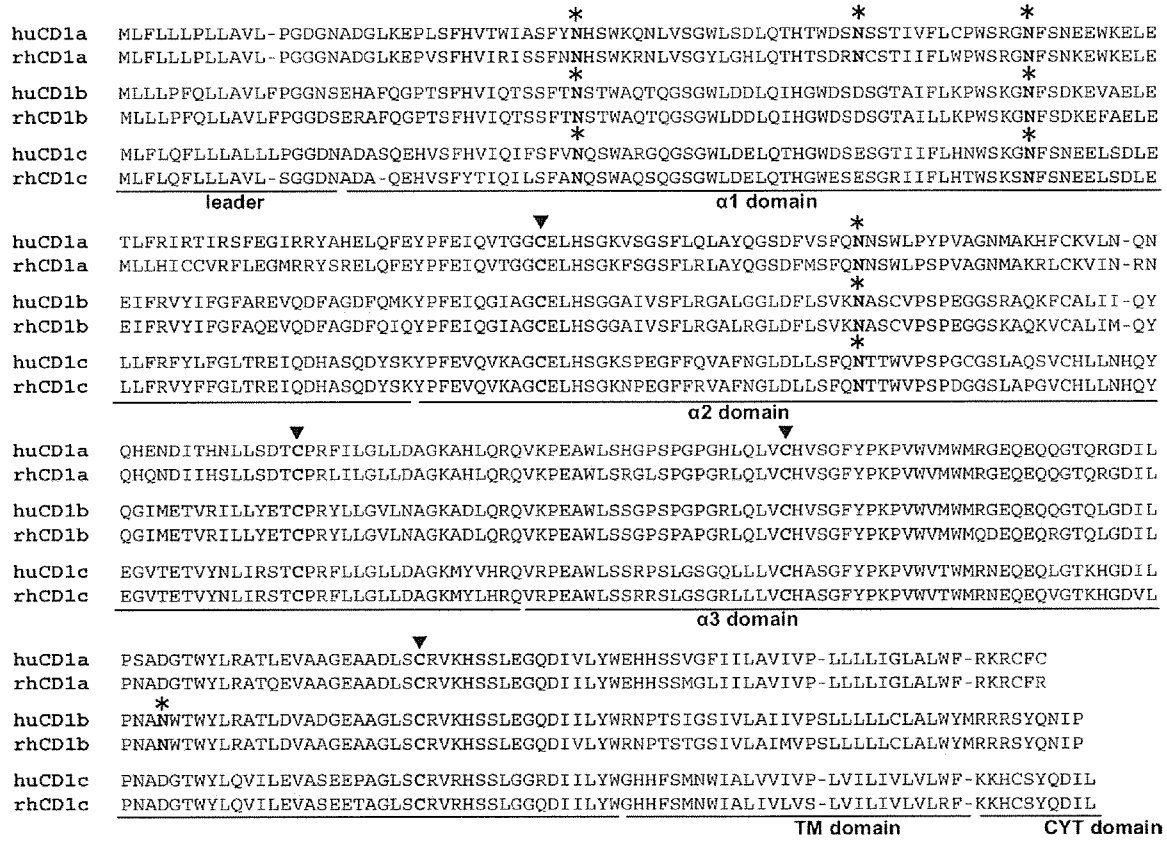

Fig. 1. Alignment of deduced amino acid sequences of human (hu) and rhesus macaque (rh) group 1 CD1 proteins. Residues conserved between the two species are shaded in light gray. Solid triangles denote cysteines conserved in all the group 1 CD1 proteins of both species that are presumed to be involved in intradomain disulfide bond formation. Asterisks indicate potential N-linked glycosylation sites. Dashes represent gaps that have been introduced to maximize alignment. TM domain, transmembrane domain; CYT domain, cytoplasmic domain.

of the putative rhesus macaque *CD1A*, *CD1B*, and *CD1C* genes into a rhesus macaque kidney epithelial cell line, LLC-MK2, and their protein expression was monitored by flow cytometry using the cross-reactive mAbs (Fig. 2B). The 10H3 anti-human CD1a mAb recognized only *CD1A* transfected cells, but not those transfected with the other genes. Similarly, the SN13 anti-human CD1b mAb and the M241 anti-human CD1c mAb showed specific reactivity to cells transfected with the *CD1B* and the *CD1C* genes, respectively. These results provided both evidence for protein expression of the isolated genes and further support for their identity, and therefore, the nucleotide sequences of the putative *CD1A*, *CD1B*, and *CD1C* cDNAs were deposited to the DDBJ/GenBank/EMBL databases as those of rhesus macaque *CD1A* (Accession Nos: AB458511), *CD1B* (AB458512), and *CD1C* (AB458513), respectively.

Trans-species activation of human T cells by rhesus macaque CD1b molecules

With the exception of mice and rats, group 1 CD1 genes have been identified in virtually all mammalian animals so far analyzed, but the Ag presentation function of their products has not been demonstrated so explicitly as in humans [21]. This is partly due to difficulties in obtaining specific T cell lines and clones that recognize lipid Ags in the context of CD1 molecules of a given animal species. Because of the highly conserved amino acid sequences of human and rhesus macaque group 1 CD1 proteins, we considered the possibility that rhesus macaque CD1 molecules might bind lipid Ags that were known to be presented by human CD1 molecules, and interact with specific human TCRs. To address this, human TCRs derived either from a dideoxymycobactin-specific, CD1a-restricted T cell line (CD8-2), from a GMM-specific, CD1b-restricted T cell line (LDN5) or from a mannosyl phosphomycoketide-specific, CD1c-restricted T cell line (CD8-1) were reconstituted in TCR-deficient Jurkat cells (J.RT3) by gene transfer, and the T cell reactivity to specific Ag in the presence of cell transfectants expressing a relevant CD1 isoform of either human or rhesus macaque origin was assessed by measuring IL-2 released from the T cells. J.RT3/CD8-2 cells responded to dideoxymycobactin in the presence of APCs expressing human CD1a molecules, but not those expressing rhesus macaque CD1a molecules (Fig. 3, top panel). Similarly, J.RT3/CD8-1 cells responded to mannosyl phosphomycoketide in the presence of APCs expressing human CD1c molecules, but not those expressing rhesus macaque CD1c molecules (bottom panel). Strikingly, however, APCs expressing rhesus macaque CD1b molecules were capable of presenting GMM efficiently to J.RT3/LDN5 cells (middle panel), providing evidence for their Ag presentation function. The apparently more efficient Ag presentation function for rhesus macaque CD1b molecules as compared with human CD1b molecules could be accounted for by the slightly higher expression on rhesus macaque CD1b transfectants than on human CD1b transfectants (data not shown).

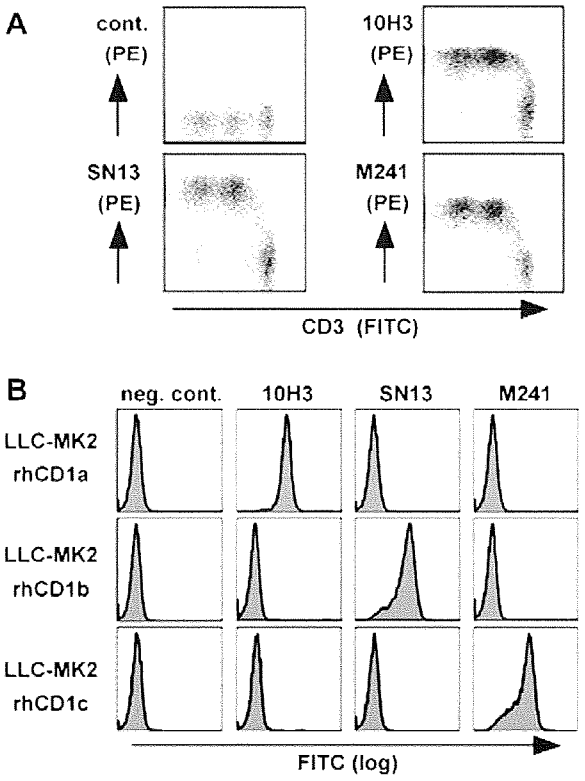

Fig. 2. Cross-reactivity of anti-human CD1 mAbs to rhesus macaque group 1 CD1 proteins. (A) Rhesus macaque thymocytes were double-labeled with the SP34 anti-CD3 mAb and either the 10H3 anti-human CD1a mAb, the SN13 anti-human CD1b mAb, the M241 anti-human CD1c mAb, or negative control Abs, followed by analysis by flow cytometry. (B) A rhesus macaque kidney cell line, LLC-MK2, that stably transfected with either rhesus macaque *CD1A* (LLC-MK2 rhCD1a), *CD1B* (LLC-MK2 rhCD1b), or *CD1C* (LLC-MK2 rhCD1c) were labeled with indicated mAbs and analyzed by flow cytometry.

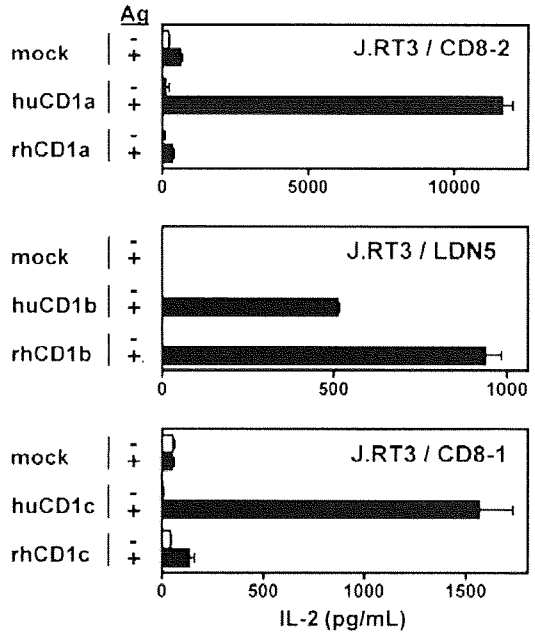

Fig. 3. Ag presentation function of rhesus macaque CD1b molecules. The J.RT3/CD8-2 cells were cultured in the presence or absence of the organic extract of *M. tuberculosis* (50 mg/ml) with T2 cells expressing either human CD1a (huCD1a) or rhesus macaque CD1a (rhCD1a) or those that were mock-transfected (top panel). The J.RT3/LDN5 cells were cultured in the presence or absence of purified GMM (5 mg/ml) with T2 cells expressing either human CD1b (huCD1b) or rhesus macaque CD1b (rhCD1b) or those that were mock transfected (middle panel). The J.RT3/CD8-1 cells were cultured in the presence or absence of the organic extract of *M. tuberculosis* (1.56 mg/ml) with HeLa cells expressing either human CD1c (huCD1c) or rhesus macaque CD1c (rhCD1c) or those that were mock transfected (bottom panel). After 20 h, the culture supernatants were harvested and the amount of IL-2 secreted into the supernatants were measured.

Trans-species crossreaction has never been observed previously for any of the group 1 CD1 molecules. Nevertheless, a molecular model of the rhesus macaque CD1b molecule has detected the α1 and α2 helix structure as well as intramolecular pockets (A′, C′, and F′) and a tunnel (T′) virtually identical to those for human CD1b molecules [22,23], allowing stable interaction with a human CD1b-presented mycobacterial Ag, GMM (Fig. 4). Further, amino acid residues, such as E80 and D83 in the α1 domain and T157 and T165 in the α2 domain, that are proposed to be critical for interaction with specific TCRs [24] are shared between rhesus macaque and human CD1b molecules, suggesting a conserved function for CD1b in these two species. The extent of amino acid sequence conservation is higher in CD1b than in CD1a and CD1c (Fig. 1), which may imply that immune responses to mycolic acid-containing glycolipds are critical for host defense against tuberculosis. So far, no experimental animals have proved extremely useful as a model for studying the group 1 CD1-mediated immunity in human infectious diseases. The present study underscores that monkeys are indispensable for a variety of challenges, including development of a new type of lipid-based vaccines against tuberculosis.

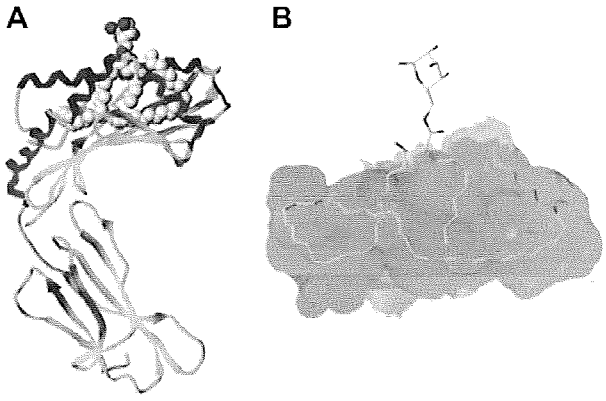

Fig. 4. A molecular model of rhesus macaque CD1b proteins. The rhesus macaque CD1b structure was constructed, based on the crystal structure of the human CD1b-GMM complex. (A) The overall structure of the rhesus macaque CD1b-GMM complex is shown, in which the CD1b heavy chain is depicted in ribbon diagram and the non-hydrogen atoms of GMM are drawn as van der Waals spheres (carbon in gray; oxygen in red). The associated β2-microglobulin is not depicted for simplicity purposes. (B) The binding surface of the Ag-binding groove is drawn in green with the bound GMM in stick (carbon in gray; oxygen in red). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this paper).

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References

[1] C.T. Morita, R.A. Mariuzza, M.B. Brenner, Antigen recognition by human gamma delta T cells: pattern recognition by the adaptive immune system, Springer Semin. Immunopathol. 22 (2000) 191–217.
[2] S.A. Porcelli, The CD1 family: a third lineage of antigen-presenting molecules, Adv. Immunol. 59 (1995) 1–98.
[3] R.J. North, Y.J. Jung, Immunity to tuberculosis, Annu. Rev. Immunol. 22 (2004) 599–623.
[4] T. Mogues, M.E. Goodrich, L. Ryan, R. LaCourse, R.J. North, The relative importance of T cell subsets in immunity and immunopathology of airborne *Mycobacterium tuberculosis* infection in mice, J. Exp. Med. 193 (2001) 271–280.
[5] C.C. Dascher, K. Hiromatsu, J.W. Naylor, P.P. Brauer, K.A. Brown, J.R. Storey, S.M. Behar, E.S. Kawasaki, S.A. Porcelli, M.B. Brenner, K.P. LeClair, Conservation of a CD1 multigene family in the guinea pig, J. Immunol. 163 (1999) 5478–5488.
[6] K. Hiromatsu, C.C. Dascher, M. Sugita, C. Gingrich-Baker, S.M. Behar, K.P. LeClair, M.B. Brenner, S.A. Porcelli, Characterization of guinea-pig group 1 CD1 proteins, Immunology 106 (2002) 159–172.
[7] D.H. Barouch, J. Kunstman, M.J. Kuroda, J.E. Schmitz, S. Santra, F.W. Peyerl, G.R. Krivulka, K. Beaudry, M.A. Lifton, D.A. Gorgone, D.C. Montefiori, M.G. Lewis, S.M. Wolinsky, N.L. Letvin, Eventual AIDS vaccine failure in a rhesus monkey by viral escape from cytotoxic T lymphocytes, Nature 415 (2002) 335–339.
[8] Y. Shen, D. Zhou, L. Qiu, X. Lai, M. Simon, L. Shen, Z. Kou, Q. Wang, L. Jiang, J. Estep, R. Hunt, M. Clagett, P.K. Sehgal, Y. Li, X. Zeng, C.T. Morita, M.B. Brenner, N.L. Letvin, Z.W. Chen, Adaptive immune response of Vgamma2Vdelta2+ T cells during mycobacterial infections, Science 295 (2002) 2255–2258.
[9] R.N. Hull, W.R. Cherry, O.J. Tritch, Growth characteristics of monkey kidney cell strains LLC-MK1, LLC-MK2, and LLC-MK2(NCTC-3196) and their utility in virus research, J. Exp. Med. 115 (1962) 903–918.
[10] M.L. Wei, P. Cresswell, HLA-A2 molecules in an antigen-processing mutant cell contain signal sequence-derived peptides, Nature 356 (1992) 443–446.
[11] M. Sugita, E.P. Grant, E. van Donselaar, V.W. Hsu, R.A. Rogers, P.J. Peters, M.B. Brenner, Separate pathways for antigen presentation by CD1 molecules, Immunity 11 (1999) 743–752.
[12] M. Sugita, X. Cao, G.F. Watts, R.A. Rogers, J.S. Bonifacino, M.B. Brenner, Failure of trafficking and antigen presentation by CD1 in AP-3-deficient cells, Immunity 16 (2002) 697–706.
[13] M. Cernadas, M. Sugita, N. van der Wel, X. Cao, J.E. Gumperz, S. Maltsev, G.S. Besra, S.M. Behar, P.J. Peters, M.B. Brenner, Lysosomal localization of murine CD1d mediated by AP-3 is necessary for NK T cell development, J. Immunol. 171 (2003) 4149–4155.
[14] H. Suzuki, M. Motohara, A. Miyake, K. Ibuki, Y. Fukazawa, K. Inaba, K. Masuda, N. Minato, H. Kawamoto, M. Hayami, T. Miura, Intrathymic effect of acute pathogenic SHIV infection on T-lineage cells in newborn macaques, Microbiol. Immunol. 49 (2005) 667–679.
[15] D. Olive, P. Dubreuil, C. Mawas, Two distinct TL-like molecular subsets defined by monoclonal antibodies on the surface of human thymocytes with different expression on leukemia lines, Immunogenetics 20 (1984) 253–264.
[16] E.P. Grant, M. Degano, J.P. Rosat, S. Stenger, R.L. Modlin, I.A. Wilson, S.A. Porcelli, M.B. Brenner, Molecular recognition of lipid antigens by T cell receptors, J. Exp. Med. 189 (1999) 195–205.
[17] C.E. Wheelock, Y. Nakagawa, T. Harada, N. Oikawa, M. Akamatsu, G. Smagghe, D. Stefanou, K. Iatrou, L. Swevers, High-throughput screening of ecdysone agonists using a reporter gene assay followed by 3-D QSAR analysis of the molting hormonal activity, Bioorg. Med. Chem. 14 (2006) 1143–1159.
[18] M. Sugita, R.M. Jackman, E. van Donselaar, S.M. Behar, R.A. Rogers, P.J. Peters, M.B. Brenner, S.A. Porcelli, Cytoplasmic tail-dependent localization of CD1b antigen-presenting molecules to MIICs, Science 273 (1996) 349–352.
[19] M. Sugita, N. Van Der Wel, R.A. Rogers, P.J. Peters, M.B. Brenner, CD1c molecules broadly survey the endocytic system, Proc. Natl. Acad. Sci. USA 97 (2000) 8445–8450.
[20] L.L. Lanier, J.P. Allison, J.H. Phillips, Correlation of cell surface antigen expression on human thymocytes by multi-color flow cytometric analysis: implications for differentiation, J. Immunol. 137 (1986) 2501–2507.
[21] K. Hiromatsu, C.C. Dascher, K.P. LeClair, M. Sugita, S.T. Furlong, M.B. Brenner, S.A. Porcelli, Induction of CD1-restricted immune responses in guinea pigs by immunization with mycobacterial lipid antigens, J. Immunol. 169 (2002) 330–339.
[22] T. Batuwangala, D. Shepherd, S.D. Gadola, K.J. Gibson, N.R. Zaccai, A.R. Fersht, G.S. Besra, V. Cerundolo, E.Y. Jones, The crystal structure of human CD1b with a bound bacterial glycolipid, J. Immunol. 172 (2004) 2382–2388.
[23] S.D. Gadola, N.R. Zaccai, K. Harlos, D. Shepherd, J.C. Castro-Palomino, G. Ritter, R.R. Schmidt, E.Y. Jones, V. Cerundolo, Structure of human CD1b with bound ligands at 2.3 A, a maze for alkyl chains, Nat. Immunol. 3 (2002) 721–726.
[24] A. Melian, G.F. Watts, A. Shamshiev, G. De Libero, A. Clatworthy, M. Vincent, M.B. Brenner, S. Behar, K. Niazi, R.L. Modlin, S. Almo, D. Ostrov, S.G. Nathenson, S.A. Porcelli, Molecular recognition of human CD1b antigen complexes: evidence for a common pattern of interaction with alpha beta TCRs, J. Immunol. 165 (2000) 4494–4504.

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Transmission of Simian Immunodeficiency Virus Carrying Multiple Cytotoxic T-Lymphocyte Escape Mutations with Diminished Replicative Ability Can Result in AIDS Progression in Rhesus Macaques[▽]

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Cytotoxic T-lymphocyte (CTL) responses frequently select for immunodeficiency virus mutations that result in escape from CTL recognition with viral fitness costs. The replication in vivo of such viruses carrying not single but multiple escape mutations in the absence of the CTL pressure has remained undetermined. Here, we have examined the replication of simian immunodeficiency virus (SIV) with five *gag* mutations selected in a macaque possessing the major histocompatibility complex haplotype *90-120-Ia* after its transmission into *90-120-Ia*-negative macaques. Our results showed that even such a "crippled" SIV infection can result in persistent viral replication, multiple reversions, and AIDS progression.

Virus-specific $CD8^+$ cytotoxic T-lymphocyte (CTL) responses exert a suppressive effect on human immunodeficiency virus (HIV) and simian immunodeficiency virus (SIV) replication (1, 10, 15, 21, 27). Under the CTL pressure, viral mutations resulting in viral escape from CTL recognition are frequently selected for, with viral fitness costs (2, 5, 8, 9, 12, 16, 19, 20, 24, 25, 26, 28). The transmission of the virus carrying a CTL escape mutation with lower viral fitness between major histocompatibility complex class I (MHC-I)-mismatched individuals can result in reversion of the mutation due to the absence of the CTL pressure (7, 14, 16, 17, 18). Such CTL escape mutations and their reversions have been suggested to be involved in viral evolution (3, 11, 13, 23).

We have developed a prophylactic vaccine using a Sendai virus vector expressing SIVmac239 Gag and shown its protective efficacy against SIVmac239 challenge in a group of Burmese rhesus macaques (*Macaca mulatta*) possessing MHC-I haplotype *90-120-Ia* (20). In these vaccinated macaques that are controlling SIVmac239 replication, $Gag_{206-216}$ epitope-specific CTL responses exerted strong selective pressure on the virus, and rapid selection of a mutant escaping from this CTL was observed at week 5 postchallenge. The virus, SIVmac 239Gag216S, with this CTL escape mutation, GagL216S, leading to a substitution from leucine (L) to serine (S) at amino acid (aa) 216 in Gag showed lower replicative ability than the wild type (14, 20). Two of these vaccinees (macaques V3 and V5) showed an accumulation of additional viral CTL escape mutations in *gag* during the period of viral control and then the reappearance of plasma viremia around week 60 after SIVmac239 challenge (12). The SIV carrying these multiple CTL escape mutations showed lower replicative ability in vitro than the SIV carrying the single GagL216S mutation.

How such viruses with multiple CTL escape mutations replicate and evolve in the absence of the CTL pressure has not yet been well determined, while the reversion of CTL escape mutations has previously been shown by the transmission of viruses with single escape mutations (7, 14, 18). In the present study, we have examined the replication, in the absence of the CTL pressure in *90-120-Ia*-negative macaques, of the SIV with multiple *gag* CTL escape mutations that were accumulated in a *90-120-Ia*-positive macaque.

The induction of $Gag_{206-216}$-specific CTL, $Gag_{241-249}$-specific CTL, and $Gag_{373-380}$-specific CTL responses has previously been observed after SIVmac239 challenge in *90-120-Ia*-positive macaques (12). The *90-120-Ia*-positive vaccinees V5 and V3 showed rapid selection of the GagL216S mutation ($Gag_{206-216}$ CTL escape mutation) and then of an additional two mutations resulting in escape from $Gag_{241-249}$-specific CTL and $Gag_{373-380}$-specific CTL recognition, respectively, during the period of viral control. These were a $Gag_{241-249}$ CTL escape mutation leading to a GagD244E (aspartic acid [D] to glutamic acid [E] at aa 244 in Gag) substitution and a $Gag_{373-380}$ CTL escape mutation leading to GagA373T (alanine [A] to threonine [T] at aa 373) in vaccinee V5 or GagV375A (valine [V] to A at aa 375) or GagP376S (proline [P] to S at aa 376) in vaccinee V3. Viruses at the reappearance of viremia had one or two additional mutations in *gag*, GagI247 L (isoleucine [I] to L at aa 247) and GagA312V (A to V at aa

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312) in vaccinee V5 or GagP172S (P to S at aa 172) or GagV145A (V to A at aa 145) in vaccinee V3. All of these mutations except for the $Gag_{373-380}$ CTL escape mutations resulted in amino acid changes in the Gag CA. We constructed molecular clones of SIVs with these *gag* mutations (12). The SIVs with three CTL escape mutations ($Gag_{206-216}$, $Gag_{241-249}$, and $Gag_{373-380}$ CTL escape mutations) were referred to as group Q SIV mutants, and the SIVs with four or five *gag* mutations selected at the reappearance of viremia as group R SIV mutants. These group Q and R SIV mutants both showed lower replicative ability in vitro than SIVmac239Gag216S, while in the competition assay between groups Q and R, the viral replicative ability was not significantly affected by the GagP172S or GagV145A mutation but was reduced by the addition of the GagI247L and GagA312V mutations (12). These results do not support the possibility of compensation for loss of viral fitness from these mutations (4, 6). In the present study, we have examined the in vivo replication of the SIV carrying five *gag* mutations, GagL216S, GagD244E, GagI247L, GagA312V, and GagA373T, selected in macaque V5 at the reappearance of viremia, which was assumed to show the lowest replicative ability among group Q and R SIV mutants. The macaques were maintained in accordance with the guidelines for animal experiments performed at the National Institute of Infectious Diseases (22).

We first compared the in vivo replication abilities of the SIV with a single GagL216S mutation and the SIVs with multiple CTL escape mutations in *90-120-Ia*-negative macaques (Fig. 1). In the competition between SIVmac239Gag216S and group Q SIV mutants, macaque R02-017 was coinoculated intramuscularly with molecular-clone DNAs of SIVmac239Gag216S and SIVmac239Gag216S244E373T and macaque R05-002 with molecular-clone DNAs of SIVmac239Gag216S and all three group Q SIV mutants. The results of the analysis of plasma viral *gag* genome sequences (Fig. 2) showed selection of SIV mac239Gag216S; i.e., all the mutations other than GagL216S became undetectable in 3 weeks postinoculation, indicating lower replicative abilities in vivo of group Q SIV mutants than of SIVmac239Gag216S, as indicated previously by in vitro competition (12). Further analysis revealed reversion of the selected GagL216S mutation to the wild-type sequence in a few months.

In the competition between SIVmac239Gag216S and group R SIV mutants, macaque R02-023, coinoculated with molecular clone DNAs of SIVmac239Gag216S and SIVmac239Gag 216S244E247L312V373T, showed selection of the former (Fig. 2). This macaque was euthanized at week 6 before exhibiting reversion of the GagL216S mutation. In macaque R02-022, coinoculated with molecular clone DNAs of SIVmac239Gag 216S and all three group R SIV mutants, almost all mutations other than GagL216S became undetectable rapidly but the GagV145A mutation was detected even at week 14. The GagL216S mutation was still dominant without reversion at week 14, and plasma viremia became undetectable after week 14 in this macaque. Both cases indicated a lower replicative ability in vivo of SIVmac239Gag216S244E247L312V373T than of SIVmac239Gag216S.

Additionally, macaque R03-022, coinoculated with the molecular-clone DNAs of SIVmac239Gag216S244E373T and SIV mac239Gag216S244E247L312V373T, showed selection of the

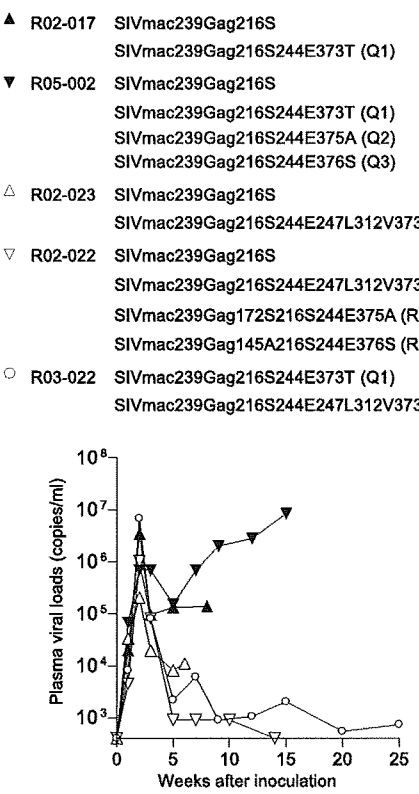

FIG. 1. Plasma viral loads of macaques used for in vivo competition assay (SIV *gag* RNA copies/ml plasma) after inoculation with SIV molecular-clone DNAs. Animals received 10 mg in total of DNAs consisting of an equal amount of each DNA; i.e., macaques R02-017, R02-023, and R03-022 were inoculated with 5 mg of each DNA, and macaques R05-002 and R02-022 with 2.5 mg of each DNA. Plasma viral loads were determined as described previously (20).

former (Fig. 2), indicating a lower replicative ability in vivo of SIVmac239Gag216S244E247L312V373T than of SIVmac239 Gag216S244E373T. In this macaque, reversion of the GagL216S mutation was observed in 6 months, while the GagD244E and GagA373T mutations were still dominant without reversion.

Next, we inoculated *90-120-Ia*-negative macaques with the SIV carrying multiple *gag* CTL escape mutations that was selected in *90-120-Ia*-positive macaque V5 (Fig. 3). The SIV carrying five gag mutations, GagL216S, GagD244E, GagI247L, GagA312V, and GagA373T, that was dominant at the reappearance of viremia in macaque V5, was propagated on rhesus macaque peripheral blood mononuclear cells to prepare the SIVmac239Gag216S244E247L312V373T challenge stock for macaques R05-001 and R06-016. Sequencing analysis confirmed no *gag* mutation except for the five mutations in the challenge virus. These two macaques were challenged intravenously with 1,000 50% tissue culture infective dose of SIVmac 239Gag216S244E247L312V373T. Both of them showed persistent viremia, although the levels of set-point plasma viral loads were low in macaque R06-016. Macaque R05-001, maintaining high viral loads, showed typical signs of AIDS, such as a reduction in peripheral $CD4^+$ T-cell counts, diarrhea, and general weakness, and was euthanized approximately 2 years postchallenge. Autopsy revealed postpersistent generalized

Macaque R02-017 inoculated with molecular clones of SIVmac239 Gag 216S & SIVmac239 Gag 216S 244E 373T

Wks	aa sequences in Gag		
p-c	**216th**	244th	373rd
1	Mt	**Wt(mt)**	**Wt(mt)**
3	Mt	**Wt**	**Wt**
8	**Wt**	**Wt**	**Wt**

Macaque R05-002 inoculated with molecular clones of SIVmac239 Gag 216S & SIVmac239 Gag 216S 244E 373T & SIVmac239 Gag 216S 244E 375A & SIVmac239 Gag 216S 244E 376S

Wks	aa sequences in Gag				
p-c	**216th**	244th	373rd	375th	376th
1	Mt	wt/mt	**Wt(mt)**	**Wt(mt)**	**Wt**
3	Mt	**Wt**	**Wt**	**Wt**	**Wt**
12	**Wt**	**Wt**	**Wt**	**Wt**	**Wt**

Macaque R02-023 inoculated with molecular clones of SIVmac239 Gag 216S & SIVmac239 Gag 216S 244E 247L 312V 373T

Wks	aa sequences in Gag				
p-c	**216th**	244th	247th	312th	373rd
1	Mt	**Wt**	**Wt**	**Wt**	**Wt(mt)**
3	Mt	**Wt**	**Wt**	**Wt**	**Wt(mt)**
6	Mt	**Wt**	**Wt**	**Wt**	**Wt**

Macaque R02-022 inoculated with molecular clones of SIVmac239 Gag 216S & SIVmac239 Gag 216S 244E 247L 312V 373T & SIVmac239 Gag 172S 216S 244E 375A & SIVmac239 Gag 145A 216S 244E 376S

Wks	aa sequences in Gag								
p-c	145th	172nd	**216th**	244th	247th	312th	373rd	375th	376th
1	**Wt(mt)**	wt/mt	Mt	wt/mt	**Wt(mt)**	**Wt(mt)**	**Wt**	wt/mt	**Wt(mt)**
3	wt/mt	**Wt**	Mt	wt/mt	**Wt**	**Wt**	**Wt**	**Wt(mt)**	wt/mt
14	wt/mt	**Wt**	Mt	**Wt**	**Wt**	**Wt**	**Wt**	**Wt**	**Wt**

Macaque R03-022 inoculated with molecular clones of SIVmac239 Gag 216S 244E 373T & SIVmac239 Gag 216S 244E 247L 312V 373T

Wks	aa sequences in Gag				
p-c	**216th**	**244th**	247th	312th	**373rd**
1	Mt	Mt	**Wt**	**Wt**	Mt
3	Mt	Mt	**Wt**	**Wt**	Mt
20	wt/mt	Mt	**Wt**	**Wt**	Mt
25	**Wt**	Mt	**Wt**	**Wt**	Mt

FIG. 2. Dominant viral genome sequences in competition assay. A *gag* DNA fragment was amplified from plasma RNA by reverse transcription and nested PCR and sequenced as described previously (20). The amino acid sequences at the positions where mutations were included in the inoculums are shown. Q and R groups of SIV mutants are described in the text. **Wt**, only the wild-type sequence was detected; **Wt**(mt), the wild-type sequence was dominant but the mutant was detectable (the mutant/wild-type ratio was less than 1/4); wt/mt, the wild type and the mutant were detected equally; Mt(wt), the mutant was dominant but the wild type was detectable (the wild-type/mutant ratio was less than 1/4); Mt, only the mutant was detected. Other than the residues indicated in this figure, no dominant mutation resulting in an amino acid change was detected in the *gag* region in macaque R02-017, R05-002, R02-023, or R02-022, but macaque R03-022 showed one amino acid change resulting in a GagV375M substitution at weeks 20 and 25. p-c, postchallenge.

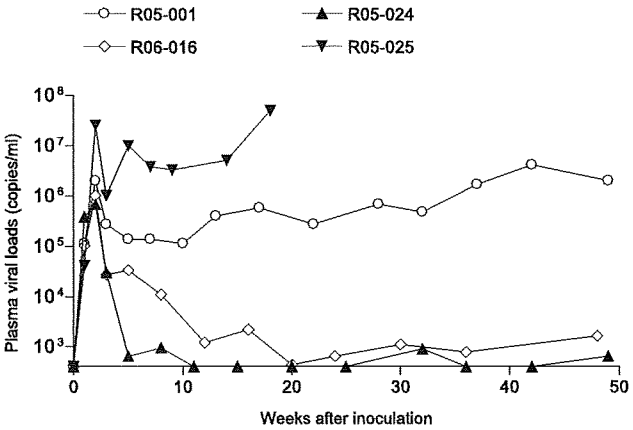

FIG. 3. Plasma viral loads (SIV *gag* RNA copies/ml plasma) in macaques after challenge with SIV carrying five *gag* mutations.

lymphadenopathy conditions and pneumocystis pneumonia. This macaque showed reversion of the GagD244E mutation in a few months, followed by reversion of the GagL216S, GagI247 L, and GagA312V mutations in a year postchallenge, while the GagA373T mutation remained dominant without reversion until euthanasia (Fig. 4). In contrast, macaque R06-016, with lower viral loads, showed no reversion of the five mutations. In the chronic phase, these two macaques showed additional Gag amino acid changes, including GagI140V (I to V at aa 140) and GagV375M (V to methionine [M] at aa 375) that were detected in both. Some of these mutations may contribute to the recovery of viral fitness.

To see the possibility of transmission of the viruses carrying the five gag mutations in the context of the polyclonal, V5-derived SIVs, macaques R05-024 and R05-025 were inoculated with plasma obtained from macaque V5 in the chronic phase of SIVmac239 infection (Fig. 3). For the challenge, plasma was obtained from macaque V5 at weeks 81, 87, 92, 100, and 113

Macaque R05-001 infected with SIVmac239Gag216S244E247L312V373T

Wks	aa sequences in Gag					
p-c	**216th**	**244th**	**247th**	**312th**	**373rd**	other residues
1	Mt	Mt	Mt	Mt	Mt	
3	Mt	Mt	Mt	Mt	Mt	
10	Mt	**Wt**	Mt	Mt	Mt	I140V
22	wt/mt	**Wt**	Mt	Mt	Mt	I140V
37	wt/mt	**Wt**	Mt	Mt	Mt	V3A, I140V
42	**Wt**(mt)	**Wt**	Mt	Mt	Mt	V3A, (V68L/M), I140V
49	**Wt**	**Wt**	wt/mt	wt/mt	Mt	V3A, (V68L/M), I140V
55	**Wt**	**Wt**	**Wt**	**Wt**	Mt	V3A, (V68L/M), I140V, (V340M), D429N
86	**Wt**	**Wt**	**Wt**	**Wt**	Mt	V3A, (V68L/M), I140V, D429N
110	**Wt**	**Wt**	**Wt**	**Wt**	Mt	V3A, (V68L/M), (I140V), V375M, (D429E)

Macaque R06-016 infected with SIVmac239Gag216S244E247L312V373T

Wks	aa sequences in Gag					
p-c	**216th**	**244th**	**247th**	**312th**	**373rd**	other residues
5	Mt	Mt	Mt	Mt	Mt	
12	Mt	Mt	Mt	Mt	Mt	
30	Mt	Mt	Mt	Mt	Mt	S128P, I140V, V375M
54	Mt	Mt	Mt	Mt	Mt	I140V, V375M

Macaque R05-024 infected with V5-plasma

Wks	aa sequences in Gag										
p-c	3rd	68th	145th	**216th**	**244th**	**247th**	**312th**	**373rd**	390th	404th	other residues
1	Mt(wt)	wt/mt	Mt	Mt	Mt	Mt	Mt	Mt	wt/mt	wt/mt	
5	Mt(wt)	wt/mt	Mt	Mt	Mt	Mt	Mt	Mt	wt/mt	wt/mt	I257K
49	Mt	Mt	Mt	Mt	Mt	**Wt**	**Wt**	**Wt**	**Wt**	Mt	A222V, I257K, R485K

Macaque R05-025 infected with V5-plasma

Wks	aa sequences in Gag										
p-c	3rd	68th	145th	**216th**	**244th**	**247th**	**312th**	**373rd**	390th	404th	other residues
1	Mt	wt/mt	Mt	Mt	Mt	Mt	Mt	Mt	wt/mt	wt/mt	
5	Mt	wt/mt	Mt	Mt	Mt	**Wt**(mt)	**Wt**(mt)	Mt	wt/mt	wt/mt	
7	Mt	wt/mt	Mt	Mt	Mt	**Wt**	**Wt**	Mt	wt/mt	wt/mt	
14	Mt	wt/mt	Mt	Mt	Mt	**Wt**	**Wt**	Mt	wt/mt	wt/mt	
18	Mt	**Wt**(mt)	Mt	Mt	Mt	**Wt**	**Wt**	Mt	wt/mt	wt/mt	

FIG. 4. Dominant viral genome sequences after challenge with SIV carrying five *gag* mutations. The amino acid sequences at the residues where mutations were included in the inoculums and dominant amino acid changes at other residues in *gag* are shown. In the column of other residues, the predominant mutations with detectable wild-type sequence are shown in parentheses. **Wt**, **Wt**(mt), wt/mt, Mt(wt), Mt, and p-c are defined in the Fig. 2 legend.

post-SIVmac239 challenge and 0.2 ml of each was intravenously inoculated into these two macaques. In the challenge SIV plasma, the five *gag* mutations (GagL216S, GagD244E, GagI247L, GagA312V, and GagA373T) and GagV145A were dominant, and additional *gag* mutations were detected in the MA- and NC-coding regions. In macaque R05-024, exhibiting low viral loads, the SIV GagL216S and GagD244E mutations remained dominant, while reversion of the GagI247L, GagA312V, and GagA373T mutations was observed (Fig. 4). Macaque R05-025, exhibiting high viral loads, developed AIDS and was euthanized at week 18 postchallenge. Autopsy revealed lymphoatrophy and cytomegalovirus infection. This macaque showed rapid reversion of the SIV GagI247L and GagA312V mutations but maintained the GagL216S, GagD 244E, and GagA373T mutations until euthanasia.

In samples from these four macaques challenged with SIV mac239Gag216S244E247L312V373T or V5-derived plasma, we examined the virus-specific $CD8^+$ T-cell responses around 3 months postinfection by flow cytometric analysis of antigen-specific gamma interferon induction (data not shown) as described previously (14, 20). Analyses using vesicular stomatitis virus G-pseudotyped SIV-infected cells as a stimulator revealed SIV-specific $CD8^+$ T-cell responses in macaques R05-001, R06-016, and R05-024, but not in macaque R05-025, which may have contributed to the rapid AIDS progression in this animal. Macaque R05-024, exhibiting lower viral loads and rapid selection of a *gag* mutation resulting in an I257K (I to lysine [K] at aa 257) substitution, showed $CD8^+$ T-cell responses specific for the $Gag_{245-269}$ peptide mixture (a mixture of $Gag_{245-260}$, $Gag_{250-265}$, and $Gag_{255-269}$ peptides), suggesting a possibility of this mutation for viral escape from strong CTL pressure. None of these four macaques showed $CD8^+$ T-cell responses specific for the $Gag_{206-225}$ (a mixture of $Gag_{206-220}$ and $Gag_{210-225}$ peptides), $Gag_{206-225}$.216S ($Gag_{206-220}$.216S and $Gag_{210-225}$.216S), $Gag_{232-255}$ ($Gag_{232-246}$, $Gag_{236-250}$, and $Gag_{240-255}$), $Gag_{232-255}$.244E, $Gag_{236-255}$.244E247L, $Gag_{362-385}$ ($Gag_{362-377}$, $Gag_{367-381}$, and $Gag_{371-385}$), or $Gag_{362-385}$.373T peptide mixture, indicating that CTL responses were not involved in the reversion or nonreversion at residue 216, 244, 247, or 373 in these macaques.

The in vivo competition assay in the present study showed loss of viral fitness from the addition of the GagD244E and GagA373T mutations into SIVmac239Gag216S and further loss of viral fitness from additional GagI247L and GagA312V mutations. The reversion of GagD244E in macaque R05-001, GagA373T in macaque R05-024, and GagI247L and GagA312V in macaques R05-024 and R05-025 (Fig. 4) supports this notion. However, reversion was not observed in all the mutations after challenge with SIV carrying the five *gag* mutations. Challenge with SIVmac239Gag216S carrying the single GagL216S mutation has shown its reversion in 3 months (14), whereas the reversion of the GagL216S mutation was

delayed or not observed after challenge with the SIV carrying five *gag* mutations. This may be due to the predominant selection of the reversion of other mutations or to lower viral replication efficiency in the latter case. Compensatory mutations can also be involved in this delay or nonreversion, but no additional *gag* mutation was observed in the early phase in macaque R06-016. The possibility of a contribution to this delay by GagI140V in macaque R05-001 and GagV145A in macaques R05-024 and R05-025 may be considered, while significant recovery of viral fitness by the latter mutation has not been observed (12).

It has been suggested that a reduction in viral fitness by CTL escape mutations may contribute to HIV/SIV control (19, 20, 28). Pressure by multiple epitope-specific CTLs may result in the selection of HIV/SIV with diminished replicative ability because of accumulating multiple escape mutations. The inefficient viral replication in macaques R02-022 and R03-022 (Fig. 1) and two of four macaques in the second experiment (Fig. 3) may reflect such a lower replicative ability of the mutant SIVs, but conversely, the results of the present study also showed efficient viral replication in macaques R05-001 and R05-025, indicating that the transmission of even such "crippled" HIV/SIV carrying multiple CTL escape mutations can result in persistent viral replication and AIDS progression. It remains unclear what host factors determined the viral replication efficiency in vivo in our study, while macaques with higher viral loads (R02-017, R05-002, R05-001, and R05-025) showed the first reversion earlier than those with lower viral loads (R02-022, R03-022, R06-016, and R05-024), suggesting an association of reversion with viral loads. Earlier reversion may result in the recovery of viral fitness, leading to higher viral loads, or conversely, higher viral loads may accelerate reversion.

Thus, our results suggest that in the transmission of HIV accumulating CTL escape mutations at the cost of viral fitness between MHC-mismatched individuals, even such crippled HIV infection can finally result in AIDS progression. Previous studies on SIVs with single CTL escape mutations showed their rapid reversion, but the present study on SIV with multiple CTL escape mutations indicates that the reversion of all the mutations was not required for the establishment of persistent viral replication or for the onset of disease. Furthermore, it suggests a possibility that CTL escape mutations resulting in viral fitness costs may not always revert rapidly even in the absence of CTL pressure after their transmission into MHC-mismatched hosts and can be transmitted further to other hosts. These results provide an important insight into HIV pathogenicity and evolution in human individuals with divergent MHC polymorphisms.

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REFERENCES

1. **Borrow, P., H. Lewicki, B. H. Hahn, G. M. Shaw, and M. B. 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.
2. **Borrow, P., H. Lewicki, X. Wei, M. S. Horwitz, N. Peffer, H. Meyers, J. A. Nelson, J. E. Gairin, B. H. Hahn, M. B. Oldstone, and G. M. Shaw.** 1997. Antiviral pressure exerted by HIV-1-specific cytotoxic T lymphocytes (CTL) during primary infection demonstrated by rapid selection of CTL escape virus. Nat. Med. **3**:205–211.
3. **Brander, C., and B. D. Walker.** 2003. Gradual adaptation of HIV to human host populations: good or bad news? Nat. Med. **9**:1359–1362.
4. **Crawford, H., J. G. Prado, A. Leslie, S. Hué, I. Honeyborne, S. Reddy, M. van der Stok, Z. Mncube, C. Brander, C. Rousseau, J. I. Mullins, R. Kaslow, P. Goepfert, S. Allen, E. Hunter, J. Mulenga, P. Kiepiela, B. D. Walker, and P. J. R. Goulder.** 2007. Compensatory mutation partially restores fitness and delays reversion of escape mutation within the immunodominant HLA-B*5703-restricted Gag epitope in chronic human immunodeficiency virus type 1 infection. J. Virol. **81**:8346–8351.
5. **Fernandez, C. S., I. Stratov, R. De Rose, K. Walsh, C. J. Dale, M. Z. Smith, M. B. Agy, S. L. Hu, K. Krebs, D. I. Watkins, D. H. O'Connor, M. P. Davenport, and S. J. Kent.** 2005. Rapid viral escape at an immunodominant simian-human immunodeficiency virus cytotoxic T-lymphocyte epitope exacts a dramatic fitness cost. J. Virol. **79**:5721–5731.
6. **Friedrich, T. C., C. A. Frye, L. J. Yant, D. H. O'Connor, N. A. Kriewaldt, M. Benson, L. Vojnov, E. J. Dodds, C. Cullen, R. Rudersdorf, A. L. Hughes, N. Wilson, and D. I. Watkins.** 2004. Extra-epitopic compensatory substitutions partially restore fitness to simian immunodeficiency virus variants that escape from an immunodominant cytotoxic T-lymphocyte response. J. Virol. **78**:2581–2585.
7. **Friedrich, T. C., E. J. Dodds, L. J. Yant, L. Vojnov, R. Rudersdorf, C. Cullen, D. T. Evans, R. C. Desrosiers, B. R. Mothe, J. Sidney, A. Sette, K. Kunstman, S. Wolinsky, M. Piatak, J. Lifson, A. L. Hughes, N. Wilson, D. H. O'Connor, and D. I. Watkins.** 2004. Reversion of CTL escape-variant immunodeficiency viruses in vivo. Nat. Med. **10**:275–281.
8. **Goulder, P. J., and D. I. Watkins.** 2004. HIV and SIV CTL escape: implications for vaccine design. Nat. Rev. Immunol. **4**:630–640.
9. **Goulder, P. J. R., R. E. Phillips, R. A. Colbert, S. McAdam, G. Ogg, M. A. Nowak, P. Giangrande, G. Luzzi, B. Morgana, A. Edwards, A. J. McMichael, and S. Rowland-Jones.** 1997. Late escape from an immunodominant cytotoxic T-lymphocyte response associated with progression to AIDS. Nat. Med. **3**:212–217.
10. **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.
11. **Kaslow, R. A., M. Carrington, R. Apple, L. Park, A. Muñoz, A. J. Saah, J. J. Goedert, C. Winkler, S. J. O'Brien, C. Rinaldo, R. Detels, W. Blattner, J. Phair, H. Erlich, and D. L. Mann.** 1996. Influence of combinations of human major histocompatibility complex genes on the course of HIV-1 infection. Nat. Med. **2**:405–411.
12. **Kawada, M., H. Igarashi, A. Takeda, T. Tsukamoto, H. Yamamoto, S. Dohki, M. Takiguchi, and T. Matano.** 2006. Involvement of multiple epitope-specific cytotoxic T-lymphocyte responses in vaccine-based control of simian immunodeficiency virus replication in rhesus macaques. J. Virol. **80**:1949–1958.
13. **Kiepiela, P., A. J. Leslie, I. Honeyborne, D. Ramduth, C. Thobakgale, S. Chetty, P. Rathnavalu, C. Moore, K. J. Pfafferott, L. Hilton, P. Zimbwa, S. Moore, T. Allen, C. Brander, M. M. Addo, M. Altfeld, I. James, S. Mallal, M. Bunce, L. D. Barber, J. Szinger, C. Day, P. Klenerman, J. Mullins, B. Korber, H. M. Coovadia, B. D. Walker, and P. J. R. Goulder.** 2004. Dominant influence of HLA-B in mediating the potential co-evolution of HIV and HLA. Nature **432**:769–775.
14. **Kobayashi, M., H. Igarashi, A. Takeda, M. Kato, and T. Matano.** 2005. Reversion in vivo after inoculation of a molecular proviral DNA clone of simian immunodeficiency virus with a cytotoxic-T-lymphocyte escape mutation. J. Virol. **79**:11529–11532.
15. **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.
16. **Leslie, A. J., K. J. Pfafferott, P. Chetty, R. Draenert, M. M. Addo, M. Feeney, Y. Tang, E. C. Holmes, T. Allen, J. G. Prado, M. Altfeld, C. Brander, C. Dixon, D. Ramduth, P. Jeena, S. A. Thomas, A. St. John, T. A. Roach, B. Kupfer, G. Luzzi, A. Edwards, G. Taylor, H. Lyall, G. Tudor-Williams, V. Novelli, J. Martinez-Picado, P. Kiepiela, B. D. Walker, and P. J. R. Goulder.** 2004. HIV evolution: CTL escape mutation and reversion after transmission. Nat. Med. **10**:282–289.
17. **Li, B., A. D. Gladden, M. Altfeld, J. M. Kaldor, D. A. Cooper, A. D. Kelleher, and T. M. Allen.** 2007. Rapid reversion of sequence polymorphisms domi-

nates early human immunodeficiency virus type 1 evolution. J. Virol. **81**:193–201.

18. **Loh, L., C. J. Batten, J. Petravic, M. P. Davenport, and S. J. Kent.** 2007. In vivo fitness costs of different Gag CD8 T-cell escape mutant simian-human immunodeficiency viruses for macaques. J. Virol. **81**:5418–5422.
19. **Martinez-Picado, J., J. G. Prado, E. E. Fry, K. Pfafferott, A. Leslie, S. Chetty, C. Thobakgale, I. Honeyborne, H. Crawford, P. Matthews, T. Pillay, C. Rousseau, J. I. Mullins, C. Brander, B. D. Walker, D. I. Stuart, P. Kiepiela, and P. Goulder.** 2006. Fitness cost of escape mutations in p24 Gag in association with control of human immunodeficiency virus type 1. J. Virol. **80**:3617–3623.
20. **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.
21. **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.
22. **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.)
23. **O'Connor, D. H., A. B. McDermott, K. C. Krebs, E. J. Dodds, J. E. Miller, E. J. Gonzalez, T. J. Jacoby, L. Yant, H. Piontkivska, R. Pantophlet, D. R. Burton, W. M. Rehrauer, N. Wilson, A. L. Hughes, and D. I. Watkins.** 2004. A dominant role for CD8$^+$-T-lymphocyte selection in simian immunodeficiency virus sequence variation. J. Virol. **78**:14012–14022.
24. **Peyerl, F. W., D. H. Barouch, W. W. Yeh, H. S. Bazick, J. Kunstman, K. J. Kunstman, S. M. Wolinsky, and N. L. Letvin.** 2003. Simian-human immunodeficiency virus escape from cytotoxic T-lymphocyte recognition at a structurally constrained epitope. J. Virol. **77**:12572–12578.
25. **Phillips, R. E., S. Rowland-Jones, D. F. Nixon, F. M. Gotch, J. P. Edwards, A. O. Ogunlesi, J. G. Elvin, J. A. Rothbard, C. R. Bangham, C. R. Rizza, and A. J. McMichael.** 1991. Human immunodeficiency virus genetic variation that can escape cytotoxic T cell recognition. Nature **354**:453–459.
26. **Price, D. A., P. J. Goulder, P. Klenerman, A. K. Sewell, P. J. Easterbrook, M. Troop, C. R. Bangham, and R. E. Phillips.** 1997. Positive selection of HIV-1 cytotoxic T lymphocyte escape variants during primary infection. Proc. Natl. Acad. Sci. USA **94**:1890–1895.
27. **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.
28. **Schneidewind, A., M. A. Brockman, R. Yang, R. I. Adam, B. Li, S. L. Gall, C. R. Rinaldo, S. L. Craggs, R. L. Allgaier, K. A. Power, T. Kuntzen, C.-S. Tung, M. X. LaBute, S. M. Mueller, T. Harrer, A. J. McMichael, P. J. R. Goulder, C. Aiken, C. Brander, A. D. Kelleher, and T. M. Allen.** 2007. Escape from the dominant HLA-B27-restricted cytotoxic T-lymphocyte response in Gag is associated with a dramatic reduction in human immunodeficiency virus type 1 replication. J. Virol. **81**:12382–12393.

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Determination of a major histocompatibility complex class I restricting simian immunodeficiency virus $Gag_{241-249}$ epitope

Several major histocompatibility complex class I (MHC-I) alleles such as *HLA-B*57* have been shown to be associated with lower viral loads and better prognosis in HIV-1 infections, and MHC-I-restricted epitope-specific effective cytotoxic T lymphocyte (CTL) responses are found to play an important role in this reduction of viral loads [1–3]. Characterization of these effective CTLs could contribute to the development of an effective AIDS vaccine.

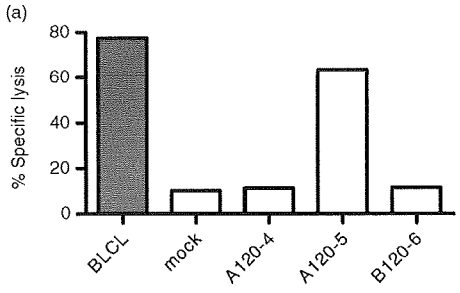
We have developed a prophylactic vaccine using a Sendai virus vector expressing simian immunodeficiency virus mac239 (SIVmac239) Gag (SeV-Gag) and have shown its protective efficacy against SIVmac239 challenge in a group of Burmese rhesus macaques (*Macaca mulatta*) sharing an MHC-I haplotype *90-120-Ia* [4]. Involvement of SIVmac239 $Gag_{241-249}$ (SSVDEQIQW) epitope-specific CTL responses in this viral control have been indicated [5]. Interestingly, the SIVmac239 $Gag_{241-249}$ epitope is located in a region corresponding to the HLA-B*57-restricted HIV-1 $Gag_{240-249}$ epitope, TW10 (TSTLQEQIAW), and TW10-specific CTL responses have also been indicated to exert strong suppressive pressure on HIV-1 replication resulting in lower viral loads [6,7]. An SIVmac239 $Gag_{241-249}$-specific CTL escape mutation has been shown to result in a loss of viral fitness similarly with a TW10-specific CTL escape mutation [5]. In the present study, for further analysis of SIVmac239 $Gag_{241-249}$-specific CTL function, we have tried to determine the MHC-I that restricts this CTL epitope.

Among eight MHC-I alleles consisting of MHC-I haplotype *90-120-Ia* [4,8], expression of three alleles, *Mamu-A*90120-4*, *Mamu-A*90120-5*, and *Mamu-B*90120-6*, was predominant at RNA levels. We cloned cDNAs of these three alleles and established HLA-A/B/C-negative human 721.221 cell lines [9] expressing these cDNAs, respectively. These cells were pulsed with 10 nmol/l of $Gag_{241-249}$ peptide and used as target cells for the CTL assay using an SIVmac239 $Gag_{241-249}$-specific CTL clone as the effector. Measurement of cytotoxicity in standard ^{51}Cr release assay [5] revealed specific killing of $Gag_{241-249}$-pulsed cells expressing Mamu-A*90120-5, indicating restriction of this CTL epitope by the Mamu-A*90120-5 molecule (Fig. 1a).

Both of the Mamu-A*90120-5-restricted SIVmac239 $Gag_{241-249}$ epitope and the HLA-B*57-restricted HIV-1 TW10 epitope are considered to have the same anchor residues, serine (S) at position 2 and tryptophan (W) at the carboxyl terminus. Comparison of amino acid sequences of antigenic peptide-binding domains (α1 and α2 domains) in Mamu-A*90120-5 with those in HLA-B*5701 revealed limited similarities (154/182 = 84.6%) between these two (Fig. 1b). This might be compatible with previous reports indicating that human and macaque MHC-I molecules with divergent peptide-binding grooves can bind similar or identical peptides [10,11]. MHC-I molecules form a peptide-binding groove including B-pocket and F-pocket that play a key role in determination of the binding peptide motif for its specific binding to the MHC-I. Mamu-A*90120-5 and HLA-B*5701 showed similarity in eight of 11 residues at 7, 9, 24, 25, 34, 45, 63, 66, 67, 70, and 99, which are considered to be anchor residues involved in B-pocket binding and in seven of eight residues at 77, 80, 81, 116, 123, 143, 146, and 147 involved in F-pocket binding [11–13].

In addition, TW10 epitope-specific CTLs, HLA-B*57-restricted HIV-1 $Gag_{147-155}$ [ISW9 (ISPRTLNAW)] epitope-specific CTLs have also been indicated to exert strong selective pressure on HIV-1 [14]. The SIVmac239 $Gag_{149-157}$ amino acid sequence corresponding to the HIV-1 $Gag_{147-155}$ epitope region is LSPRTLNAW, showing a difference at the amino terminus, and CTL responses specific for a peptide including the SIVmac239 $Gag_{149-157}$ amino acid sequence were not induced by SeV-Gag vaccination in Mamu-A*90120-5-positive macaques (data not shown). Interestingly, the SIVmac239 Gag 148th proline (P) and 149th leucine (L) correspond to the HIV-1 Gag 146th P and the 147th L, respectively that have been indicated to be selected in HIV-1-infected humans possessing HLA-B*57. Selection of the former 146th P has been shown to result in escape from ISW9-specific CTL recognition by disturbance in antigen processing [14]. Thus, it is speculated that the SIVmac239 $Gag_{149-157}$-derived peptide may not be presented by Mamu-A*90120-5 even if it has an ability to bind this peptide.

Both SIVmac239 $Gag_{241-249}$-specific CTLs and HIV-1 TW10-specific CTLs have been indicated to exert strong suppressive pressure on SIV/HIV-1 replication and select for a mutation resulting in escape from their recognition at the cost of viral fitness. Thus, this Gag region may be a promising CTL target for viral control, and SIVmac239 infection in Mamu-A*90120-5-positive macaques could be a unique model for examining viral replication in the



(b)

```
                  * *                    **         *
Mamu-A*90120-5  GSHSMRYFYT SMSRPGRGQP RFFAVGYVDD TQFVRFDSDA
HLA-B*5701      ---------- A-------E- --I------- ----------

                    *                    *  **  *        #  #
Mamu-A*90120-5  ESPRMEPRAP WVEQEGPEYW DQETRKAKDT AQNYRVNLRT
HLA-B*5701      A----A---- -I-------- -G---NM-AS --T--E---I

                #                   *                    #
Mamu-A*90120-5  ALRYYNQSEA GSHTIQKMYG CDLGPDGRLL RGYDQSAYDG
HLA-B*5701      ---------- ---I--V--- --V------- --H-------

                  #                      #  ##
Mamu-A*90120-5  RDYIALNEDL RSWTAADMAA QNTQRKWEAA GWTEQMRAYL
HLA-B*5701      K--------- S------T-- -I-------- RVA--L----

Mamu-A*90120-5  EGECLEWLRR YLENGKETLQ RA
HLA-B*5701      --L-V----- ---------- --
```

Fig. 1. Mamu-A*90120-5 that restricts the SIV $Gag_{241-249}$ epitope. (a) CTL assay using a $Gag_{241-249}$-specific CTL clone on a B-lymphoblastoid cell line derived from a macaque possessing *90-120-Ia* (BLCL), 721.221 cells (mock), and 721.221 cells expressing Mamu-A*90120-4 (A120-4), Mamu-A*90120-5 (A120-5), and Mamu-B*90120-6 (B120-6), respectively. (b) Amino acid sequences of the Mamu-A*90120-5 α1 and α2 domains in comparison with HLA-B*5701. The anchor residues involved in B and F-pocket binding are indicated by * and #, respectively.

presence of those CTLs targeting this region like TW10-specific CTLs. Finally, we obtained a phycoerythrin-conjugated $Gag_{241-249}$ epitope-Mamu-A*90120-5 tetramer for specific detection of $Gag_{241-249}$-specific CTLs. This could be useful for the analysis of $Gag_{241-249}$-specific CTL responses in Mamu-A*90120-5-positive macaques infected with SIVmac239.

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References

1. Kaslow RA, Carrington M, Apple R, Park L, Muñoz A, Saah AJ, *et al.* **Influence of combinations of human major histocompatibility complex genes on the course of HIV-1 infection.** *Nat Med* 1996; **2**:405–411.
2. Migueles SA, Sabbaghian MS, Shupert WL, Bettinotti MP, Marincola FM, Martino L, *et al.* **HLA B*5701 is highly associated with restriction of virus replication in a subgroup of HIV-infected long term nonprogressors.** *Proc Natl Acad Sci U S A* 2000; **97**:2709–2714.
3. Kiepiela P, Leslie AJ, Honeyborne I, Ramduth D, Thobakgale C, Chetty S, *et al.* **Dominant influence of HLA-B in mediating the potential co-evolution of HIV and HLA.** *Nature* 2004; **432**:769–775.
4. Matano T, Kobayashi M, Igarashi H, Takeda A, Nakamura H, Kano M, *et al.* **Cytotoxic T lymphocyte-based control of simian immunodeficiency virus replication in a preclinical AIDS vaccine trial.** *J Exp Med* 2004; **199**:1709–1718.
5. Kawada M, Igarashi H, Takeda A, Tsukamoto T, Yamamoto H, Dohki S, *et al.* **Involvement of multiple epitope-specific cytotoxic T-lymphocyte responses in vaccine-based control of simian immunodeficiency virus replication in rhesus macaques.** *J Virol* 2006; **80**:1949–1958.
6. Leslie AJ, Pfafferott KJ, Chetty P, Draenert R, Addo MM, Feeney M, *et al.* **HIV evolution: CTL escape mutation and reversion after transmission.** *Nat Med* 2004; **10**:282–289.
7. Goulder PJ, Watkins DI. **HIV and SIV CTL escape: implications for vaccine design.** *Nat Rev Immunol* 2004; **4**:630–640.
8. Takahashi-Tanaka Y, Yasunami M, Naruse T, Hinohara K, Matano T, Mori K, *et al.* **Reference strand-mediated conformation analysis (RSCA)-based typing of multiple alleles in the rhesus macaque MHC class I Mamu-A and Mamu-B loci.** *Electrophoresis* 2007; **28**:918–924.
9. Shimizu Y, DeMars R. **Production of human cells expressing individual transferred HLA-A,-B,-C genes using an HLA-A,-B,-C null human cell line.** *J Immunol* 1989; **142**:3320–3328.
10. Evans DT, Knapp LA, Jing P, Piekarczyk MS, Hinshaw VS, Watkins DI. **Three different MHC class I molecules bind the same CTL epitope of the influenza virus in a primate species with limited MHC class I diversity.** *J Immunol* 1999; **162**:3970–3977.
11. Hickman-Miller HD, Bardet W, Gilb A, Luis AD, Jackson KW, Watkins DI, *et al.* **Rhesus macaque MHC class I molecules present HLA-B-like peptides.** *J Immunol* 2005; **175**:367–375.
12. Bjorkman PJ, Saper MA, Samraoui B, Bennett WS, Strominger JL, Wiley DC. **The foreign antigen binding site and T cell recognition regions of class I histocompatibility antigens.** *Nature* 1987; **329**:512–518.
13. Saper MA, Bjorkman PJ, Wiley DC. **Refined structure of the human histocompatibility antigen HLA-A2 at 2.6 Å resolution.** *J Mol Biol* 1991; **219**:277–319.
14. Draenert R, Le Gall S, Pfafferott KJ, Leslie AJ, Chetty P, Brander C, *et al.* **Immune selection for altered antigen processing leads to cytotoxic T lymphocyte escape in chronic HIV-1 infection.** *J Exp Med* 2004; **199**:905–915.

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REVIEW

Host factors involved in resistance to retroviral infection

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ABSTRACT

Viral replication requires the help 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, which is termed intrinsic immunity and is distinguished from conventional innate and adaptive immunity, has been described as playing an important role in making species-specific barriers against viral infection. Here, we describe the current progress in understanding of such restriction factors against retroviral replication, focusing on TRIM5α and APOBEC, whose anti-retroviral effects have recently been recognized. Additionally, we mention cyclophilin A, which 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.

Key words apolipoprotein B mRNA-editing enzyme-catalytic subunit, cyclophilin A, retrovirus, tripartite interaction motif 5 α.

Among host factors exerting inhibitory effects on viral replication, the *Fv-1* and the *Fv-4* loci have been known to confer resistance to Friend virus infection in mice and their restriction mechanisms have been intensively investigated (1–5). The latter, *Fv-4*, corresponds to a defective endogenous provirus with an ecotropic MLV-like *env* gene. Entry of ecotropic MLV into cells expressing *Fv-4* is inhibited by binding of the *Fv-4 env* gene product, Fv-4 Env, to the ecotropic MLV receptor, and this receptor interference has been reported to play a central role in resistance to Friend virus infection (6). Additionally, it has recently been suggested that the Fv-4 Env-mediated dominant negative effect on MLV Env function contributes to efficient resistance in *Fv-4*-expressing mice (7). On the other hand, MLV restriction mediated by the *Fv-1* gene product, Fv-1, occurs after viral entry into the cells but before the integration step during the viral replication cycle (8). The two main alleles of *Fv-1*, *Fv-1*^{n} and *Fv-1*^{b}, confer resistance to replication of B-tropic and N-tropic MLV respectively (9). Although the precise mechanism of Fv-1-mediated restriction remains unclear, comparison of viral genome sequences between B-tropic and N-tropic MLV has indicated the 110th amino acid in Gag CA as the viral determinant for the tropism, suggesting that MLV Gag CA is the target for the host factor involved in this restriction (10, 11).

Ref-1, which shows a similar pattern of inhibition of retroviral replication with Fv-1, is known as a restriction factor in mammalian cells apart from murine cells. The viral determinant for Ref-1-mediated restriction has been

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List of Abbreviations: A, adenine; agmApo3G, African green monkey Apo3G; Ala, alanine; Apo3G, APOBEC3G; APOBEC, apolipoprotein B mRNA-editing enzyme-catalytic subunit; CA, capsid; CypA, cyclophilin A; CsA, cyclosporine A; G, guanine; Gly, glycine; hApo3G, human Apo3G; HIV-1, human immunodeficiency virus type 1; Ile, isoleucine; Lv-1, lentivirus susceptibility factor 1; MLV, murine leukemia virus; OWM, old world monkey; Pro, proline; Ref-1, restriction factor 1; SIVagm, African green monkey simian immunodeficiency virus; SIVmac, simian immunodeficiency virus mac; TRIM5, tripartite interaction motif 5; TRIM5α, tripartite interaction motif 5 α; Vif, virus infectivity factor.

reported to be at the Gag CA residue, corresponding to the 110th in MLV Gag CA involved in Fv-1-mediated restriction (12). Ref-1 and Fv-1 both show viral restriction post-viral entry in the early phase of the retroviral replication cycle, but the exact point of restriction has been indicated to be different: the former, Ref-1-mediated restriction, occurs at the step prior to reverse transcription, while the latter, Fv-1 mediated restriction, is considered to occur post-reverse transcription (8). Further, restriction of HIV-1 replication post-viral entry has been reported in OWM cells that support efficient replication of SIVmac, and the existence of a restriction factor, Lv-1, responsible for this resistance of non-human primate cells to HIV-1 replication has been suggested (13–15).

TRIM5α: A HOST FACTOR RESTRICTING HIV-1 REPLICATION POST-VIRAL ENTRY IN PRIMATE CELLS

Recently, two independent groups have identified the α-isoform of TRIM5, TRIM5α, as a restriction factor responsible for resistance of monkey cells to HIV-1 infection and shown that restriction of HIV-1 replication by TRIM5α derived from rhesus and owl monkeys but not efficiently from humans (16, 17) (Fig. 1). Subsequent studies have revealed virus-specific restriction activities by TRIM5α and its homologues derived from humans and non-human primates (18–24). For instance, restriction by rhesus monkey TRIM5α is efficient against HIV-1 but inefficient against SIVmac and undetectable against MLV (Fig. 1).

TRIM5α is a trimeric cytoplasmic protein (25, 26) consisting of RING finger, B-box, coiled-coil, and SPRY (B30.2) domains (27, 28). The coiled-coil domain is indispensable for TRIM5α multimerization, and both the coiled-coil and the SPRY domains are required for its binding to the virion core (28, 29).

TRIM5α-midiated restriction of HIV-1 replication is considered to occur after viral entry in the early phase of the viral replication cycle, but its precise mechanism remains unclear and several possibilities have been proposed. First, it has been suggested that binding of TRIM5α to the virion CA after viral entry may accelerate or abrogate the process of its uncoating and disruption, resulting in inhibition of HIV-1 replication (29). Second, involvement of ubiquitin in TRIM5α-mediated restriction has been suggested by recent reports showing that a mutation in its RING finger domain decreases the restriction ability of TRIM5α (16, 30–32) and that recovery from the restriction occurs in the presence of proteosome inhibitors (32, 33), although this is controversial (29, 31, 32). Furthermore, some reports have shown TRIM5α-mediated

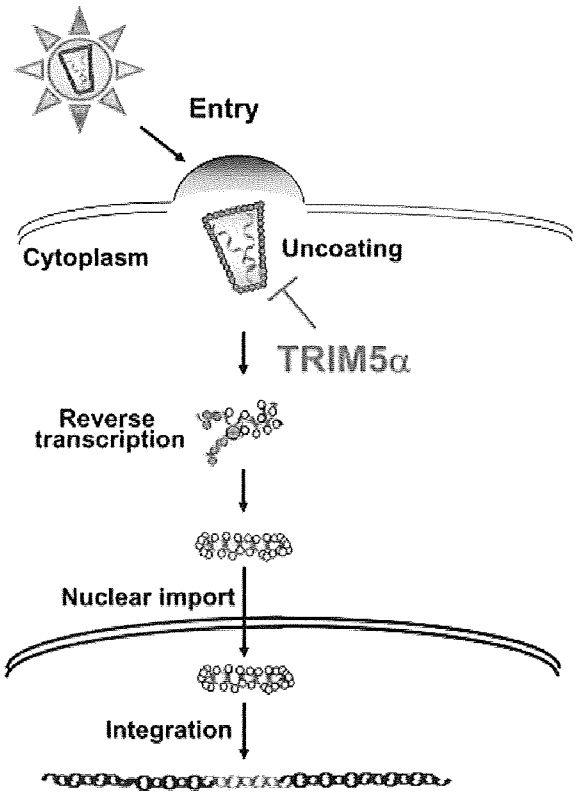

Fig. 1. A schema for TRIM5α-mediated restriction of HIV-1 replication in OWM cells. Recognition of HIV-1 CA by TRIM5α results in restriction of HIV-1 replication at the step after viral entry into the cytoplasm.

inhibition of viral cDNA nuclear import as well as viral cDNA synthesis (34, 35). In addition to restriction at the early phase of retroviral replication cycle, TRIM5α has recently been shown to inhibit virus production by accelerating degradation of viral Gag protein (36).

APOBEC: AN ENZYMATIC HOST FACTOR RESTRICTING RETROVIRAL REPLICATION

HIV-1 replication in primary CD4$^+$ T lymphocytes and monocytes is dependent on the presence of an HIV-1 accessory protein, Vif, which has been reported to work in a host cell-specific manner (37, 38). Vif is required for infectious HIV-1 production from some immortalized human T cell lines such as CEM (termed non-permissive) but not in others such as CEM-SS (termed permissive), and the existence of a restriction factor whose anti-retroviral activity can be abrogated by Vif has been suggested in the case of the non-permissive cells (39–45). Comparison of the non-permissive and permissive cells has

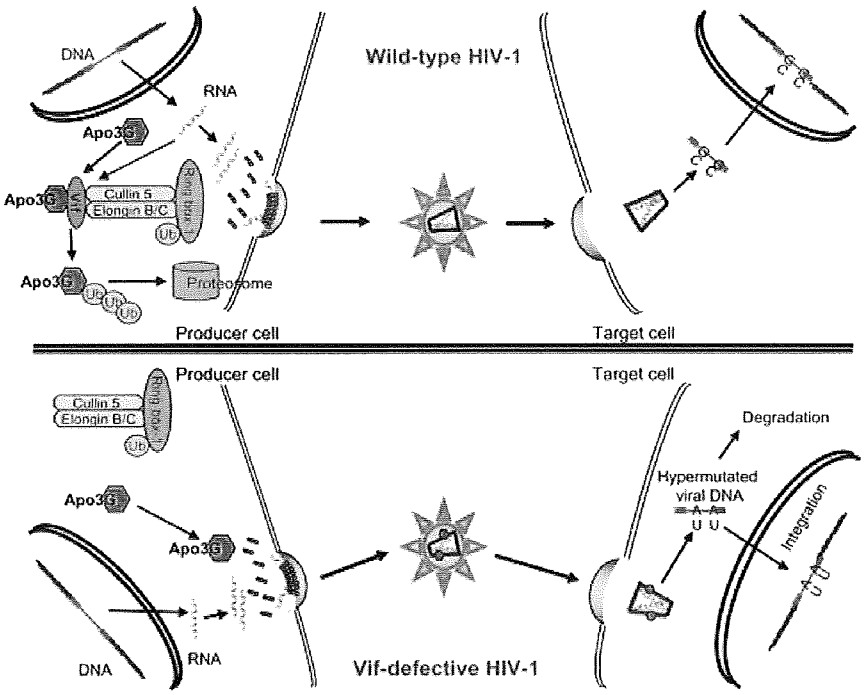

Fig. 2. A putative model for APOBEC3G-mediated restriction of HIV-1 replication and Vif-mediated recovery from the restriction. In the wild-type HIV-1 replication (upper panel), Vif connects hApo3G to an E3 ubiquitin ligase complex including Elongin B/C, Cullin5, and Ring-box-1 to induce polyubiquitylation and proteosomal degradation of hApo3G, resulting in exclusion of hApo3G from the virion with viral genome remaining intact even after viral entry. In contrast, in *vif*-deleted HIV-1 replication (lower panel), hApo3G is incorporated into the virion and its replication ability is abrogated after viral entry into the cells. Thereafter, these C-to-U mutations in the viral minus-strand DNA result in G-to-A mutations in the complementary plus-strand DNA during reverse transcription.

revealed Apo3G, a member of the APOBEC family of cytidine deaminases, to be the restriction factor responsible for inhibition of *vif*-deleted HIV-1 replication in human non-permissive cells (46). Unlike TRIM5α and Fv-1, the target of Apo3G-mediated restriction is not viral CA, but viral single-stranded cDNA synthesized during reverse transcription. It is packaged into virus particles produced from Apo3G-expressing cells and inhibits viral replication after viral entry into the cells (Fig. 2). HIV-1 Vif can inhibit the uptake of Apo3G into the virion by inducing polyubiquitylation and proteosomal degradation of cellular Apo3G, resulting in abrogation of Apo3G-mediated restriction (47) (Fig. 2).

Several mechanisms for Apo3G-mediated restriction against HIV-1 infection have been reported. First, it has been reported that the cytidine deaminase activity of Apo3G can induce hypermutation (a large number of G-to-A substitutions) in proviral DNA during reverse transcription, resulting in failure of infectious HIV-1 production (48–53) (Fig. 2). Second, the possibility of Apo3G-mediated inhibition of tRNA annealing or processing during reverse transcription has been shown (54–56). Additional mechanisms, including inhibition at the step of viral plus-strand cDNA transfer, have also been suggested (56–58).

Restriction of retroviral infection by Apo3G derived from non-human species has also been reported (51, 59–62), and the Vif-Apo3G interaction is considered to be species-specific (51, 63). Indeed, it has been indicated that hApo3G is insensitive to SIVagm Vif while agmApo3G is insensitive to HIV-1 Vif, and that the determinant for this specificity is at the 128th residue in Apo3G (51, 64–67). However, a recent report has shown that SIVagm Vif can support SIVagm replication in an hApo3G-positive human T cell line (A3.01): *vif*-deleted SIVagm replication was severely restricted with accumulation of G-to-A mutations in the viral genome, suggesting ambiguity of species specificity (68).

Restriction of HIV-1 and SIV replication by other members of the APOBEC family has been reported, although it might not be as efficient as Apo3G. Thus, APOBEC proteins are now considered to be a new class of host restriction factors against retroviral replication (61, 69). For instance, human APOBEC3F can inhibit HIV-1 replication in the absence of Vif (59, 60, 70, 71) whereas human APOBEC3B does so even in the presence of

Vif (59, 72, 73). Association of deaminase activity with Apo3G-mediated restriction of HIV-1 replication has been strongly suspected, but the possibility of involvement of deaminase activity-independent mechanisms in this restriction has also been suggested (57, 58). Indeed, several groups have reported Apo3G and APOBEC3F variants lacking in deaminase activity without loss of restriction activity, as well as variants lacking in restriction activity without loss of deaminase activity (74–77). Additionally, a recent report has suggested a deaminase-independent inhibitory effect of Apo3G on viral DNA synthesis following reverse transcription (78). However, this possibility is controversial (79, 80), and the precise mechanism for Apo3G-mediated restriction of HIV-1 infection remains unclear.

CYCLOPHILIN A: A HOST FACTOR INVOLVED IN RETROVIRAL REPLICATION

CypA, a ubiquitous protein, was first identified as the target of CsA, an immunosuppressive reagent (81). CypA has proline-isomerase activity that catalyzes *cis-trans* isomerization of the Pro residue (82, 83). CsA binding to CypA inhibits this isomerase activity (83). CypA binding to HIV-1 Gag CA has been shown by analysis using the yeast two-hybrid system (84). The Ala-Gly-Pro-Ile residues from the 88th to the 91st in CA are the key portion for its binding to the active site of CypA (85–87). Interestingly, the peptidyl-prolyl bond between the 89th Gly (Gly89) and the 90th Pro (Pro90) exhibits the *trans* conformation, (in contrast to the *cis* conformation usually observed in other known CypA targets) (87, 88), and this Pro90 residue but not other Pro is considered to be critical for the binding of CA to CypA. Thus, it has been suggested that CypA acts as a molecular chaperone without exerting *cis-trans* isomerase activity on HIV-1 CA (87). However, this is still controversial and the possibility of CypA-mediated *cis-trans* isomerization of the Gly89-Pro90 peptidyl-prolyl bond has also been suggested (88).

It has been well established that CypA promotes HIV-1 replication after viral entry in the early phase in human cells (85, 89–95) (Fig. 3). CypA is efficiently incorporated into the virion produced from HIV-1-infected cells through interaction with CA in the context of Gag polyprotein (Fig. 3). Disruption of CypA incorporation

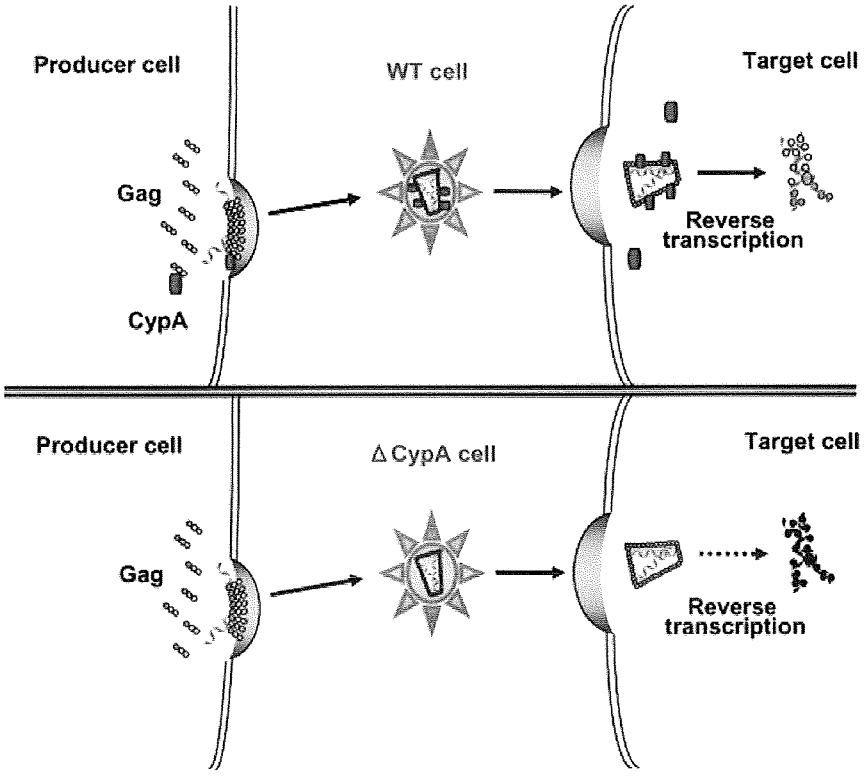

Fig. 3. A putative mechanism for CypA-mediated enhancement of HIV-1 replication. CypA is required for efficient reverse transcription (upper panel), and HIV-1 infection in CypA-deficient human cells shows inefficient reverse transcription (lower panel).

into the virion by CsA administration or by Gag mutations resulted in reduction in infectivity of the produced viruses (85, 89, 91, 95–98). Several reports have shown that both CA dimerization and CypA multimerization are required for the efficient CA-CypA binding which is critical for HIV-1 infectivity (99, 100). Recently, promotion of HIV-1 replication by post-entry interaction of CA with CypA in target cells has been shown, suggesting the importance of CypA for efficient HIV-1 replication (94, 101, 102).

Only retroviruses with CA capable of binding to CypA exhibit CypA-dependent viral replication (84, 85, 90, 92, 95). This suggests involvement of CA-CypA interaction in the determination of retroviral tropism (14, 16, 17, 34, 98, 101–111). The effect of CypA on SIV replication in human cells has not been clearly determined but, for the first time, a recent study has shown that human CypA exerts an inhibitory effect on *vif*-deleted SIV replication, which may be recovered by SIV Vif excluding the CypA from the virion (112). This Vif function can be distinguished from the anti-hApo3G function of Vif described above.

PERSPECTIVES: HOST FACTORS INVOLVED IN THE POST-ENTRY STEP OF THE EARLY PHASE OF RETROVIRAL REPLICATION

Restriction of HIV-1 replication in non-human primate cells after viral entry occurs at the step prior to reverse transcription, and TRIM5α plays a crucial role in this restriction (13–15, 101, 106, 113–115). Interestingly, CypA-dependency is considered to occur at the same step in the retroviral replication cycle (116).

Several groups have reported modest restriction of HIV-1 replication by human TRIM5α, a restriction which is not altered by disruption of the CA-CypA interaction or by elimination of endogenous CypA (117–119). In contrast, restriction of HIV-1 replication by the TRIM5α derived from OWM (such as rhesus macaques) is abrogated by CsA-mediated or small interfering RNA-mediated inhibition of OWM-derived CypA function, indicating involvement of CypA in TRIM5α-mediated restriction of HIV-1 replication in OWM cells (117, 118, 120). In the owl monkey (a new world monkey), a CypA-TRIM5α-fusion protein has been found and involvement of CypA in the restriction has been suggested (17). Thus, CypA may exert restriction activity against HIV-1 replication in association with TRIM5α in non-human primates but not in humans. Elucidation of the key factors involved in this difference in CypA function between non-human primates and humans may contribute to understanding of the species-specific restriction mechanism against retroviral replication.

REFERENCES

1. Suzuki S. (1975) FV-4: a new gene affecting the splenomegaly induction by Friend leukemia virus. *Jpn J Exp Med* **45:** 473–478.
2. Gardner M.B., Rasheed S., Pal B.K., Estes J.D., O'Brien S.J. (1980) Akvr-1, a dominant murine leukemia virus restriction gene, is polymorphic in leukemia-prone wild mice. *Proc Natl Acad Sci USA* **77:** 531–535.
3. Rasheed S., Gardner M.B. (1983) Resistance to fibroblasts and hematopoietic cells to ecotropic murine leukemia virus infection; an Akvr-1R gene effect. *Int J Cancer* **31:** 491–496.
4. Hartley J.W., Rowe W.P., Huebner R.J. (1970) Host-range restrictions of murine leukemia viruses in mouse embryo cell cultures. *J Virol* **5:** 221–225.
5. Pincus T., Hartley J.W., Rowe W.P. (1971) A major genetic locus affecting resistance to infection with murine leukemia viruses. I. Tissue culture studies of naturally occurring viruses. *J Exp Med* **133:** 1219–1233.
6. Ikeda H., Odaka T. (1983) Cellular expression of murine leukemia virus gp70-related antigen on thymocytes of uninfected mice correlates with Fv-4 gene-controlled resistance to Friend leukemia virus infection. *Virology* **128:** 127–139.
7. Takeda A., Matano T. (2007) Inhibition of infectious murine leukemia virus production by Fv-4 env gene products exerting dominant negative effect on viral envelope glycoprotein. *Microbes Infect.* **9:**1590–6.
8. Goff S.P. (2004) Genetic control of retrovirus susceptibility in mammalian cells. *Annu Rev Genet* **38:** 61–85.
9. Rein A., Kashmiri S.V., Bassin R.H., Gerwin B.L., Duran-Troise G. (1976) Phenotypic mixing between N- and B-tropic murine leukemia viruses: infectious particles with dual sensitivity to Fv-1 restriction. *Cell* **7:** 373–379.
10. Gautsch J.W., Elder J.H., Schindler J., Jensen F.C., Lerner R.A. (1978) Structural markers on core protein p30 of murine leukemia virus: functional correlation with Fv-1 tropism. *Proc Natl Acad Sci USA* **75:** 4170–4174.
11. Kozak C.A., 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.
12. Towers G., Bock M., Martin S., Takeuchi Y., Stoye J.P., Danos O. (2000) A conserved mechanism of retrovirus restriction in mammals. *Proc Natl Acad Sci USA* **97:** 12 295–12 299.
13. Besnier C., Takeuchi Y., Towers G. (2002) Restriction of lentivirus in monkeys. *Proc Natl Acad Sci USA* **99:** 11 920–11 925.
14. Cowan S., Hatziioannou T., Cunningham T., Muesing M.A., Gottlinger H.G., Bieniasz P.D. (2002) Cellular inhibitors with Fv1-like activity restrict human and simian immunodeficiency virus tropism. *Proc Natl Acad Sci USA* **99:** 11 914–11 919.
15. Munk C., Brandt S.M., Lucero G., Landau N.R. (2002) A dominant block to HIV-1 replication at reverse transcription in simian cells. *Proc Natl Acad Sci USA* **99:** 13 843–13 848.
16. Stremlau M., Owens C.M., Perron M.J., Kiessling M., Autissier P., Sodroski J. (2004) The cytoplasmic body component TRIM5alpha restricts HIV-1 infection in Old World monkeys. *Nature* **427:** 848–853.
17. Sayah D.M., Sokolskaja E., Berthoux L., Luban J. (2004) Cyclophilin A retrotransposition into TRIM5 explains owl monkey resistance to HIV-1. *Nature* **430:** 569–573.
18. Keckesova Z., Ylinen L.M., Towers G.J. (2004) The human and African green monkey TRIM5alpha genes encode Ref1 and Lv1 retroviral restriction factor activities. *Proc Natl Acad Sci USA* **101:** 10 780–10 785.
19. Perron M.J., Stremlau M., Song B., Ulm W., Mulligan R.C., Sodroski J. (2004) TRIM5alpha mediates the postentry block to

N-tropic murine leukemia viruses in human cells. *Proc Natl Acad Sci USA* **101:** 11 827–11 832.
20. Si Z., Vandegraaff N., O'Huigin C., Song B., Yuan W., Xu C., Perron M., Li X., Marasco W.A., Engelman A., *et al.* (2006) Evolution of a cytoplasmic tripartite motif (TRIM) protein in cows that restricts retroviral infection. *Proc Natl Acad Sci USA* **103:** 7454–7459.
21. Song B., Javanbakht H., Perron M., Park D.H., Stremlau M., Sodroski J. (2005) Retrovirus restriction by TRIM5alpha variants from Old World and New World primates. *J Virol* **79:** 3930–3937.
22. Yap M.W., Nisole S., Lynch C., Stoye J.P. (2004) Trim5alpha protein restricts both HIV-1 and murine leukemia virus. *Proc Natl Acad Sci USA* **101:** 10 786–10 791.
23. Ylinen L.M., Keckesova Z., Wilson S.J., Ranasinghe S., Towers G.J. (2005) Differential restriction of human immunodeficiency virus type 2 and simian immunodeficiency virus SIVmac by TRIM5alpha alleles. *J Virol* **79:** 11 580–11 587.
24. Hatziioannou T., Perez-Caballero D., Yang A., Cowan S., Bieniasz P.D. (2004) Retrovirus resistance factors Ref1 and Lv1 are species-specific variants of TRIM5alpha. *Proc Natl Acad Sci USA* **101:** 10 774–10 779.
25. Mische C.C., Javanbakht H., Song B., Diaz-Griffero F., Stremlau M., Strack B., Si Z., Sodroski J. (2005) Retroviral restriction factor TRIM5alpha is a trimer. *J Virol* **79:** 14 446–14 450.
26. Javanbakht H., Yuan W., Yeung D.F., Song B., Diaz-Griffero F., Li Y., Li X., Stremlau M., Sodroski J. (2006) Characterization of TRIM5alpha trimerization and its contribution to human immunodeficiency virus capsid binding. *Virology* **353:** 234–246.
27. Nisole S., Stoye J.P., Saib A. (2005) TRIM family proteins: retroviral restriction and antiviral defence. *Nat Rev Microbiol* **3:** 799–808.
28. Reymond A., Meroni G., Fantozzi A., Merla G., Cairo S., Luzi L., Riganelli D., Zanaria E., Messali S., Cainarca S., *et al.* (2001) The tripartite motif family identifies cell compartments. *Embo J* **20:** 2140–2151.
29. Stremlau M., Perron M., Lee M., Li Y., Song B., Javanbakht H., Diaz-Griffero F., Anderson D.J., Sundquist W.I., Sodroski J. (2006) Specific recognition and accelerated uncoating of retroviral capsids by the TRIM5alpha restriction factor. *Proc Natl Acad Sci USA* **103:** 5514–5519.
30. Perez-Caballero D., Hatziioannou T., Yang A., Cowan S., Bieniasz P.D. (2005) Human tripartite motif 5alpha domains responsible for retrovirus restriction activity and specificity. *J Virol* **79:** 8969–8978.
31. Javanbakht H., Diaz-Griffero F., Stremlau M., Si Z., Sodroski J. (2005) The contribution of RING and B-box 2 domains to retroviral restriction mediated by monkey TRIM5alpha. *J Biol Chem* **280:** 26 933–26 940.
32. Perez-Caballero D., Hatziioannou T., Zhang F., Cowan S., Bieniasz P.D. (2005) Restriction of human immunodeficiency virus type 1 by TRIM-CypA occurs with rapid kinetics and independently of cytoplasmic bodies, ubiquitin, and proteasome activity. *J Virol* **79:** 15 567–15 572.
33. Schwartz O., Marechal V., Friguet B., Arenzana-Seisdedos F., Heard J.M. (1998) Antiviral activity of the proteasome on incoming human immunodeficiency virus type 1. *J Virol* **72:** 3845–3850.
34. Berthoux L., Sebastian S., Sokolskaja E., Luban J. (2004) Lv1 inhibition of human immunodeficiency virus type 1 is counteracted by factors that stimulate synthesis or nuclear translocation of viral cDNA. *J Virol* **78:** 11 739–11 750.
35. Wu X., Anderson J.L., Campbell E.M., Joseph A.M., Hope T.J. (2006) Proteasome inhibitors uncouple rhesus TRIM5alpha restriction of HIV-1 reverse transcription and infection. *Proc Natl Acad Sci USA* **103:** 7465–7470.
36. Sakuma R., Noser J.A., Ohmine S., Ikeda Y. (2007) Rhesus monkey TRIM5alpha restricts HIV-1 production through rapid degradation of viral Gag polyproteins. *Nat Med* **13:** 631–635.
37. Fisher A.G., Ensoli B., Ivanoff L., Chamberlain M., Petteway S., Ratner L., Gallo R.C., Wong-Staal F. (1987) The sor gene of HIV-1 is required for efficient virus transmission in vitro. *Science* **237:** 888–893.
38. Strebel K., Daugherty D., Clouse K., Cohen D., Folks T., Martin M.A. (1987) The HIV 'A' (sor) gene product is essential for virus infectivity. *Nature* **328:** 728–730.
39. Akari H., Sakuragi J., Takebe Y., Tomonaga K., Kawamura M., Fukasawa M., Miura T., Shinjo T., Hayami M. (1992) Biological characterization of human immunodeficiency virus type 1 and type 2 mutants in human peripheral blood mononuclear cells. *Arch Virol* **123:** 157–167.
40. Blanc D., Patience C., Schulz T.F., Weiss R., 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.
41. Borman A.M., Quillent C., Charneau P., Dauguet C., Clavel F. (1995) Human immunodeficiency virus type 1 Vif- mutant particles from restrictive cells: role of Vif in correct particle assembly and infectivity. *J Virol* **69:** 2058–2067.
42. Fan L., Peden K. (1992) Cell-free transmission of Vif mutants of HIV-1. *Virology* **190:** 19–29.
43. Gabuzda D.H., Lawrence K., Langhoff E., Terwilliger E., Dorfman T., Haseltine W.A., Sodroski J. (1992) Role of vif in replication of human immunodeficiency virus type 1 in CD4+ T lymphocytes. *J Virol* **66:** 6489–6495.
44. Sakai H., Shibata R., Sakuragi J., Sakuragi S., Kawamura M., Adachi A. (1993) Cell-dependent requirement of human immunodeficien-
cy virus type 1 Vif protein for maturation of virus particles. *J Virol* **67:** 1663–1666.
45. von Schwedler U., Song J., Aiken C., Trono D. (1993) Vif is crucial for human immunodeficiency virus type 1 proviral DNA synthesis in infected cells. *J Virol* **67:** 4945–4955.
46. Sheehy A.M., Gaddis N.C., Choi J.D., Malim M.H. (2002) Isolation of a human gene that inhibits HIV-1 infection and is suppressed by the viral Vif protein. *Nature* **418:** 646–650.
47. Yu X., Yu Y., Liu B., Luo K., Kong W., Mao P., Yu X.F. (2003) Induction of APOBEC3G ubiquitination and degradation by an HIV-1 Vif-Cul5-SCF complex. *Science* **302:** 1056–1060.
48. Harris R.S., Bishop K.N., Sheehy A.M., Craig H.M., Petersen-Mahrt S.K., Watt I.N., Neuberger M.S., Malim M.H. (2003) DNA deamination mediates innate immunity to retroviral infection. *Cell* **113:** 803–809.
49. Lecossier D., Bouchonnet F., Clavel F., Hance A.J. (2003) Hypermutation of HIV-1 DNA in the absence of the Vif protein. *Science* **300:** 1112.
50. Mangeat B., Turelli P., Caron G., Friedli M., Perrin L., Trono D. (2003) Broad antiretroviral defence by human APOBEC3G through lethal editing of nascent reverse transcripts. *Nature* **424:** 99–103.
51. Mariani R., Chen D., Schrofelbauer B., Navarro F., Konig R., Bollman B., Munk C., Nymark-McMahon H., Landau N.R. (2003) Species-specific exclusion of APOBEC3G from HIV-1 virions by Vif. *Cell* **114:** 21–31.
52. Yu Q., Konig R., Pillai S., Chiles K., Kearney M., Palmer S., Richman D., Coffin J.M., Landau N.R. (2004) Single-strand specificity of APOBEC3G accounts for minus-strand deamination of the HIV genome. *Nat Struct Mol Biol* **11:** 435–442.

53. Zhang H., Yang B., Pomerantz R.J., Zhang C., Arunachalam S.C., Gao L. (2003) The cytidine deaminase CEM15 induces hypermutation in newly synthesized HIV-1 DNA. *Nature* **424:** 94–98.
54. Guo F., Cen S., Niu M., Saadatmand J., Kleiman L. (2006) Inhibition of formula-primed reverse transcription by human APOBEC3G during human immunodeficiency virus type 1 replication. *J Virol* **80:** 11 710–11 722.
55. Guo F., Cen S., Niu M., Yang Y., Gorelick R.J., Kleiman L. (2007) The interaction of APOBEC3G with human immunodeficiency virus type 1 nucleocapsid inhibits tRNA3Lys annealing to viral RNA. *J Virol* **81:** 11 322–11 331.
56. 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 immunodeficiency virus type 1 cDNAs produced in the presence of APOBEC3G exhibit defects in plus-strand DNA transfer and integration. *J Virol* **81:** 7099–7110.
57. Li X.Y., Guo F., Zhang L., Kleiman L., Cen S. (2007) APOBEC3G inhibits DNA strand transfer during HIV-1 reverse transcription. *J Biol Chem* **282:** 32 065–32 074.
58. Luo K., Wang T., Liu B., Tian C., Xiao Z., Kappes J., Yu X.F. (2007) Cytidine deaminases APOBEC3G and APOBEC3F interact with human immunodeficiency virus type 1 integrase and inhibit proviral DNA formation. *J Virol* **81:** 7238–7248.
59. Bishop K.N., Holmes R.K., Sheehy A.M., Davidson N.O., Cho S.J., Malim M.H. (2004) Cytidine deamination of retroviral DNA by diverse APOBEC proteins. *Curr Biol* **14:** 1392–1396.
60. Wiegand H.L., Doehle B.P., Bogerd H.P., Cullen B.R. (2004) A second human antiretroviral factor, APOBEC3F, is suppressed by the HIV-1 and HIV-2 Vif proteins. *Embo J* **23:** 2451–2458.
61. Cullen B.R. (2006) Role and mechanism of action of the APOBEC3 family of antiretroviral resistance factors. *J Virol* **80:** 1067–1076.
62. Bishop K.N., Holmes R.K., Sheehy A.M., Malim M.H. (2004) APOBEC-mediated editing of viral RNA. *Science* **305:** 645.
63. Simon J.H., Miller D.L., Fouchier R.A., Soares M.A., Peden K.W., Malim M.H. (1998) The regulation of primate immunodeficiency virus infectivity by Vif is cell species restricted: a role for Vif in determining virus host range and cross-species transmission. *Embo J* **17:** 1259–1267.
64. Bogerd H.P., Doehle B.P., Wiegand H.L., 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. *Proc Natl Acad Sci USA* **101:** 3770–3774.
65. Mangeat B., Turelli P., Liao S., Trono D. (2004) A single amino acid determinant governs the species-specific sensitivity of APOBEC3G to Vif action. *J Biol Chem* **279:** 14 481–14 483.
66. Schrofelbauer B., Chen D., Landau N.R. (2004) A single amino acid of APOBEC3G controls its species-specific interaction with virion infectivity factor (Vif). *Proc Natl Acad Sci USA* **101:** 3927–3932.
67. Xu H., Svarovskaia E.S., Barr R., Zhang Y., Khan M.A., Strebel K., Pathak V.K. (2004) A single amino acid substitution in human APOBEC3G antiretroviral enzyme confers resistance to HIV-1 virion infectivity factor-induced depletion. *Proc Natl Acad Sci USA* **101:** 5652–5657.
68. Takeuchi H., Kao S., Miyagi E., Khan M.A., Buckler-White A., Plishka R., Strebel K. (2005) Production of infectious SIVagm from human cells requires functional inactivation but not viral exclusion of human APOBEC3G. *J Biol Chem* **280:** 375–382.
69. Harris R.S., Liddament M.T. (2004) Retroviral restriction by APOBEC proteins. *Nat Rev Immunol* **4:** 868–877.
70. Liddament M.T., Brown W.L., Schumacher A.J., Harris R.S. (2004) APOBEC3F properties and hypermutation preferences indicate activity against HIV-1 in vivo. *Curr Biol* **14:** 1385–1391.
71. Zheng Y.H., Irwin D., Kurosu T., Tokunaga K., Sata T., Peterlin B.M. (2004) Human APOBEC3F is another host factor that blocks human immunodeficiency virus type 1 replication. *J Virol* **78:** 6073–6076.
72. Doehle B.P., Schafer A., 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.
73. Rose K.M., Marin M., Kozak S.L., Kabat D. (2005) Regulated production and anti-HIV type 1 activities of cytidine deaminases APOBEC3B, 3F, and 3G. *AIDS Res Hum Retroviruses* **21:** 611–619.
74. Bishop K.N., Holmes R.K., Malim M.H. (2006) Antiviral potency of APOBEC proteins does not correlate with cytidine deamination. *J Virol* **80:** 8450–8458.
75. Holmes R.K., Koning F.A., Bishop K.N., Malim M.H. (2007) APOBEC3F can inhibit the accumulation of HIV-1 reverse transcription products in the absence of hypermutation. Comparisons with APOBEC3G. *J Biol Chem* **282:** 2587–2595.
76. Newman E.N., Holmes R.K., Craig H.M., Klein K.C., Lingappa J.R., Malim M.H., Sheehy A.M. (2005) Antiviral function of APOBEC3G can be dissociated from cytidine deaminase activity. *Curr Biol* **15:** 166–170.
77. Shindo K., Takaori-Kondo A., Kobayashi M., Abudu A., Fukunaga K., Uchiyama T. (2003) 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* **278:** 44 412–44 416.
78. Iwatani Y., Chan D.S., Wang F., Maynard K.S., Sugiura W., Gronenborn A.M., Rouzina I., Williams M.C., Musier-Forsyth K., Levin J.G. (2007) Deaminase-independent inhibition of HIV-1 reverse transcription by APOBEC3G. *Nucleic Acids Res.* 35(21):7096-108
79. Opi S., Takeuchi H., Kao S., Khan M.A., Miyagi E., Goila-Gaur R., Iwatani Y., Levin J.G., Strebel K. (2006) Monomeric APOBEC3G is catalytically active and has antiviral activity. *J Virol* **80:** 4673–4682.
80. Miyagi E., Opi S., Takeuchi H., Khan M., Goila-Gaur R., Kao S., Strebel K. (2007) Enzymatically active APOBEC3G is required for efficient inhibition of human immunodeficiency virus type 1. *J Virol* **81:** 13 346–13 353.
81. Handschumacher R.E., Harding M.W., Rice J., Drugge R.J., Speicher D.W. (1984) Cyclophilin: a specific cytosolic binding protein for cyclosporin A. *Science* **226:** 544–547.
82. Fischer G., Wittmann-Liebold B., Lang K., Kiefhaber T., Schmid F.X. (1989) Cyclophilin and peptidyl-prolyl cis-trans isomerase are probably identical proteins. *Nature* **337:** 476–478.
83. Takahashi N., Hayano T., Suzuki M. (1989) Peptidyl-prolyl cis-trans isomerase is the cyclosporin A-binding protein cyclophilin. *Nature* **337:** 473–475.
84. Luban J., Bossolt K.L., Franke E.K., Kalpana G.V., Goff S.P. (1993) Human immunodeficiency virus type 1 Gag protein binds to cyclophilins A and B. *Cell* **73:** 1067–1078.
85. Franke E.K., Yuan H.E., Luban J. (1994) Specific incorporation of cyclophilin A into HIV-1 virions. *Nature* **372:** 359–362.
86. Gamble T.R., Vajdos F.F., Yoo S., Worthylake D.K., Houseweart M., Sundquist W.I., Hill C.P. (1996) Crystal structure of human cyclophilin A bound to the amino-terminal domain of HIV-1 capsid. *Cell* **87:** 1285–1294.
87. Zhao Y., Chen Y., Schutkowski M., Fischer G., Ke H. (1997) Cyclophilin A complexed with a fragment of HIV-1 gag protein: insights into HIV-1 infectious activity. *Structure* **5:** 139–146.
88. Bosco D.A., Eisenmesser E.Z., Pochapsky S., Sundquist W.I., Kern D. (2002) Catalysis of cis/trans isomerization in native HIV-1 capsid by human cyclophilin A. *Proc Natl Acad Sci USA* **99:** 5247–5252.

89. Braaten D., Aberham C., Franke E.K., Yin L., Phares W., Luban J. (1996) Cyclosporine A-resistant human immunodeficiency virus type 1 mutants demonstrate that Gag encodes the functional target of cyclophilin A. *J Virol* **70:** 5170–5176.
90. Braaten D., Franke E.K., Luban J. (1996) 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. *J Virol* **70:** 4220–4227.
91. Braaten D., Luban J. (2001) Cyclophilin A regulates HIV-1 infectivity, as demonstrated by gene targeting in human T cells. *Embo J* **20:** 1300–1309.
92. Franke E.K., 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.
93. Hatziioannou T., Perez-Caballero D., Cowan S., Bieniasz P.D. (2005) Cyclophilin interactions with incoming human immunodeficiency virus type 1 capsids with opposing effects on infectivity in human cells. *J Virol* **79:** 176–183.
94. Sokolskaja E., Sayah D.M., Luban J. (2004) Target cell cyclophilin A modulates human immunodeficiency virus type 1 infectivity. *J Virol* **78:** 12 800–12 808.
95. Thali M., Bukovsky A., Kondo E., Rosenwirth B., Walsh C.T., Sodroski J., Gottlinger H.G. (1994) Functional association of cyclophilin A with HIV-1 virions. *Nature* **372:** 363–365.
96. Ott D.E., Coren L.V., Johnson D.G., Sowder R.C., 2nd, Arthur L.O., Henderson L.E. (1995) Analysis and localization of cyclophilin A found in the virions of human immunodeficiency virus type 1 MN strain. *AIDS Res Hum Retroviruses* **11:** 1003–1006.
97. Ackerson B., Rey O., Canon J., 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. *J Virol* **72:** 303–308.
98. Bukovsky A.A., Weimann A., Accola M.A., 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. *Proc Natl Acad Sci USA* **94:** 10 943–10 948.
99. Colgan J., Yuan H.E., Franke E.K., 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. *J Virol* **70:** 4299–4310.
100. Javanbakht H., Diaz-Griffero F., Yuan W., Yeung D.F., Li X., Song B., Sodroski J. (2007) The ability of multimerized cyclophilin A to restrict retrovirus infection. *Virology* **367:** 19–29.
101. Towers G.J., Hatziioannou T., Cowan S., Goff S.P., Luban J., Bieniasz P.D. (2003) Cyclophilin A modulates the sensitivity of H.I.V-1 to host restriction factors. *Nat Med* **9:** 1138–1143.
102. Kootstra N.A., Munk C., Tonnu N., Landau N.R., Verma I.M. (2003) Abrogation of postentry restriction of HIV-1-based lentiviral vector transduction in simian cells. *Proc Natl Acad Sci USA* **100:** 1298–1303.
103. Kamada K., Igarashi T., Martin M.A., Khamsri B., Hatcho K., Yamashita T., Fujita M., Uchiyama T., Adachi A. (2006) Generation of HIV-1 derivatives that productively infect macaque monkey lymphoid cells. *Proc Natl Acad Sci USA* **103:** 16 959–16 964.
104. Hatziioannou T., Princiotta M., Piatak M., Jr., Yuan F., Zhang F., Lifson J.D., Bieniasz P.D. (2006) Generation of simian-tropic HIV-1 by restriction factor evasion. *Science* **314:** 95.
105. Shibata R., Kawamura M., Sakai H., Hayami M., Ishimoto A., Adachi A. (1991) Generation of a chimeric human and simian immunodeficiency virus infectious to monkey peripheral blood mononuclear cells. *J Virol* **65:** 3514–3520.
106. Shibata R., Sakai H., Kawamura M., Tokunaga K., Adachi A. (1995) Early replication block of human immunodeficiency virus type 1 in monkey cells. *J Gen Virol* 76(Pt 11): 2723–2730.
107. Dorfman T., Gottlinger H.G. (1996) The human immunodeficiency virus type 1 capsid p2 domain confers sensitivity to the cyclophilin-binding drug SDZ NIM 811. *J Virol* **70:** 5751–5757.
108. Owens C.M., Yang P.C., Gottlinger H., Sodroski J. (2003) Human and simian immunodeficiency virus capsid proteins are major viral determinants of early, postentry replication blocks in simian cells. *J Virol* **77:** 726–731.
109. Hatziioannou T., Cowan S., Von Schwedler U.K., Sundquist W.I., Bieniasz P.D. (2004) Species-specific tropism determinants in the human immunodeficiency virus type 1 capsid. *J Virol* **78:** 6005–6012.
110. Owens C.M., Song B., Perron M.J., Yang P.C., Stremlau M., Sodroski J. (2004) Binding and susceptibility to postentry restriction factors in monkey cells are specified by distinct regions of the human immunodeficiency virus type 1 capsid. *J Virol* **78:** 5423–5437.
111. Ikeda Y., Ylinen L.M., Kahar-Bador M., Towers G.J. (2004) Influence of gag on human immunodeficiency virus type 1 species-specific tropism. *J Virol* **78:** 11 816–11 822.
112. Takeuchi H., Buckler-White A., Goila-Gaur R., Miyagi E., Khan M.A., Opi S., Kao S., Sokolskaja E., Pertel T., Luban J., *et al.* (2007) Vif counteracts a cyclophilin A-imposed inhibition of simian immunodeficiency viruses in human cells. *J Virol* **81:** 8080–8090.
113. Himathongkham S., Luciw P.A. (1996) Restriction of HIV-1 (subtype B) replication at the entry step in rhesus macaque cells. *Virology* **219:** 485–488.
114. Hofmann W., Schubert D., LaBonte J., Munson L., Gibson S., Scammell J., Ferrigno P., Sodroski J. (1999) Species-specific, postentry barriers to primate immunodeficiency virus infection. *J Virol* **73:** 10 020–10 028.
115. Hatziioannou T., Cowan S., Goff S.P., Bieniasz P.D., Towers G.J. (2003) Restriction of multiple divergent retroviruses by Lv1 and Ref1. *Embo J* **22:** 385–394.
116. Braaten D., Franke E.K., Luban J. (1996) Cyclophilin A is required for an early step in the life cycle of human immunodeficiency virus type 1 before the initiation of reverse transcription. *J Virol* **70:** 3551–3560.
117. Keckesova Z., Ylinen L.M., Towers G.J. (2006) Cyclophilin A renders human immunodeficiency virus type 1 sensitive to Old World monkey but not human TRIM5 alpha antiviral activity. *J Virol* **80:** 4683–4690.
118. Stremlau M., Song B., Javanbakht H., Perron M., Sodroski J. (2006) Cyclophilin A: an auxiliary but not necessary cofactor for TRIM5alpha restriction of HIV-1. *Virology* **351:** 112–120.
119. Sokolskaja E., Berthoux L., Luban J. (2006) Cyclophilin A and TRIM5alpha independently regulate human immunodeficiency virus type 1 infectivity in human cells. *J Virol* **80:** 2855–2862.
120. Berthoux L., Sebastian S., Sokolskaja E., Luban J. (2005) Cyclophilin A is required for TRIM5{alpha}-mediated resistance to HIV-1 in Old World monkey cells. *Proc Natl Acad Sci USA* **102:** 14 849–14 853.

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Gag-Specific Cytotoxic T-Lymphocyte-Based Control of Primary Simian Immunodeficiency Virus Replication in a Vaccine Trial[▽]

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Gag-specific cytotoxic T lymphocytes (CTLs) exert strong suppressive pressure on human immunodeficiency virus (HIV) and simian immunodeficiency virus (SIV) replication. However, it has remained unclear whether they can actually contain primary viral replication. Recent trials of prophylactic vaccines inducing virus-specific T-cell responses have indicated their potential to confer resistance against primary SIV replication in rhesus macaques, while the immunological determinant for this vaccine-based viral control has not been elucidated thus far. Here we present evidence implicating Gag-specific CTLs as responsible for the vaccine-based primary SIV control. Prophylactic vaccination using a Gag-expressing Sendai virus vector resulted in containment of SIVmac239 challenge in all rhesus macaques possessing the major histocompatibility complex (MHC) haplotype *90-120-Ia*. In contrast, *90-120-Ia*-positive vaccinees failed to contain SIVs carrying multiple *gag* CTL escape mutations that had been selected, at the cost of viral fitness, in SIVmac239-infected *90-120-Ia*-positive macaques. These results show that Gag-specific CTL responses do play a crucial role in the control of wild-type SIVmac239 replication in vaccinees. This study implies the possibility of Gag-specific CTL-based primary HIV containment by prophylactic vaccination, although it also suggests that CTL-based AIDS vaccine efficacy may be abrogated in viral transmission between MHC-matched individuals.

Despite tremendous efforts to develop AIDS vaccines eliciting virus-specific T-cell responses, whether this approach actually does result in controlling human immunodeficiency virus (HIV) replication remains unknown. Recent trials have shown reductions in postchallenge viral loads by prophylactic vaccination eliciting virus-specific T-cell responses in macaque AIDS models (19, 22, 34), but the first advanced human trial of a T-cell-based vaccine was halted because of a lack of efficacy (5). Hence, it is quite important to determine which T-cell responses are responsible for primary HIV control.

Cytotoxic T-lymphocyte (CTL) responses have been indicated to play an important role in the control of HIV and simian immunodeficiency virus (SIV) infections (2, 9, 10, 17, 23, 29). Above all, the potential of Gag-specific CTL responses to contribute to viral control has been suggested by a cohort study indicating an association of HIV control with the breadth of Gag-specific CTL responses (15). In support of this, a recent in vitro study revealed their ability to rapidly respond to SIV infection (28). However, it has remained unclear whether Gag-specific CTL-based viral containment can be achieved by prophylactic vaccination.

We previously developed a prophylactic AIDS vaccine regimen consisting of a DNA prime followed by a boost with a Sendai virus (SeV) vector expressing SIVmac239 Gag (SeV-Gag) (22, 32). Our trial showed potential for efficiently inducing Gag-specific T-cell responses and containment of SIVmac239 challenge in a group of Burmese rhesus macaques sharing the major histocompatibility complex class I (MHC-I) haplotype *90-120-Ia* (22). A follow-up study revealed the reappearance of plasma viremia at >1 year postchallenge in some of these *90-120-Ia*-positive SIV controllers. In these transient controllers, multiple CTL escape mutations were accumulated in the viral *gag* gene, resulting in viremia reappearance and thus suggesting the involvement of $Gag_{206-216}$ (IINEEAADWDL) epitope-specific, $Gag_{241-249}$ (SSVDEQIQW) epitope-specific, and $Gag_{373-380}$ (APVPIPFA) epitope-specific CTLs in sustained viral control (12). Nonetheless, it has remained undetermined whether such Gag-specific CTL responses were responsible for the vaccine-based primary SIV control in *90-120-Ia*-positive vaccinees. In the present study, we challenged the *90-120-Ia*-positive vaccinees with SIVs carrying the *gag* CTL escape mutations to determine the role of Gag-specific CTLs in primary SIVmac239 control.

MATERIALS AND METHODS

Viral competition assay. SIV molecular clone DNAs with *gag* mutations were constructed by site-directed mutagenesis from the wild-type SIVmac239 (14) molecular clone DNA. Virus stocks were obtained by transfection of COS-1 cells with wild-type or mutant SIV molecular clone DNAs, and their titers were

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