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.

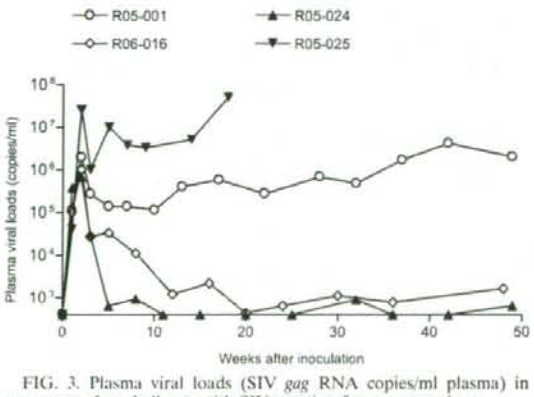

FIG. 3. Plasma viral loads (SIV *gag* RNA copies/ml plasma) in macaques after challenge with SIV carrying five *gag* mutations.

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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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

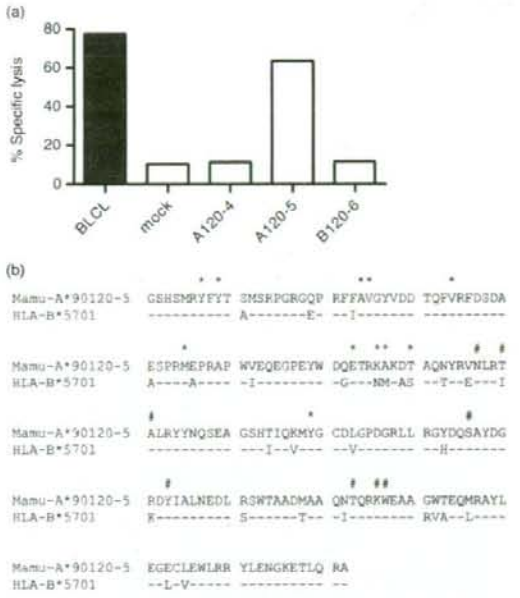

Fig. 1. Mamu-A*90120-5 that restricts the SIV $Gag_{241-249}$ epitope. (a) CTL assay using a $Gag_{241-249}$-specific CTL clone on a B-lymphoblastoid cell line derived from a macaque possessing *90-120-Ia* (BLCL), 721.221 cells (mock), and 721.221 cells expressing Mamu-A*90120-4 (A120-4), Mamu-A*90120-5 (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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Antigen-specific T-cell induction by vaccination with a recombinant Sendai virus vector even in the presence of vector-specific neutralizing antibodies in rhesus macaques

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ABSTRACT

Recombinant viral vectors are promising vaccine tools for eliciting potent cellular immune responses against immunodeficiency virus infection, but pre-existing anti-vector antibodies can be an obstacle to their clinical use in humans. We have previously vaccinated rhesus macaques with a recombinant Sendai virus (SeV) vector twice at an interval of more than 1 year and have shown efficient antigen-specific T-cell induction by the second as well as the first vaccination. Here, we have established the method for measurement of SeV-specific neutralizing titers and have found efficient SeV-specific neutralizing antibody responses just before the second SeV vaccination in these macaques. This suggests the feasibility of inducing antigen-specific T-cell responses by SeV vaccination even in the host with pre-existing anti-SeV neutralizing antibodies.

Virus-specific $CD8^+$ cytotoxic T lymphocyte (CTL) responses are crucial for the control of human immunodeficiency virus (HIV) and simian immunodeficiency virus (SIV) replication [1–5]. Efficient CTL induction is a key strategy for AIDS vaccine development, and recombinant viral vectors are promising tools for its elicitation [6]. Most of the parental or related viruses of these vectors can induce natural infection in humans. Thus, pre-existing immunity against the vector virus itself could be an obstacle to viral vector-based efficient CTL induction in humans. Indeed, a clinical trial of a vaccine using adenovirus serotype 5 (AdV5) vectors has shown reduction in efficiency of vaccine-based CTL induction in people with pre-existing anti-AdV5 antibodies [7–9].

We have developed an AIDS vaccine using a recombinant Sendai virus (SeV) vector and have shown its potential for efficient CTL induction in macaques [10–12]. SeV, murine parainfluenza virus type 1 (PIV-1), is an enveloped virus with a negative-sense RNA genome. Its natural host is mice and its natural infection is not believed to occur efficiently in primates including humans [11,13]. However, antibodies against human PIV-1 (hPIV-1), whose natural infection frequently occurs in humans, are known to cross-react with SeV [14]. It can be expected that the presence of these cross-reactive antibodies at SeV vaccination may reduce its efficiency of CTL induction, but it remains unclear how much extent anti-hPIV-1 antibodies may have adverse effect on SeV-based CTL induction in humans.

Recently, we have vaccinated four rhesus macaques with SeV vectors twice at an interval of more than 1 year and examined antigen-specific CTL induction by the second SeV vaccination [15]. The second vaccination of macaques with an SIV Gag-expressing SeV (SeV-Gag) vector resulted in efficient induction of Gag-specific CTL responses. In the present study, we have established the method for measurement of SeV-specific neutralizing titers and examined SeV-specific neutralizing antibody responses at the second SeV-Gag vaccination in these four macaques. Our results revealed that Gag-specific CTL responses were induced by the second SeV-Gag vaccination in the presence of anti-SeV neutralizing antibodies.

Materials and methods

Samples. Plasma samples were obtained from four Burmese rhesus macaques (*Macaca mulatta*), R011, R012, R003, and R006, that received SeV vaccination twice as described previously [15]. In brief, macaques R011 and R012 received four times of vaccinations with an env- and nef-deleted simian–human immunodeficiency virus (SHIV) molecular clone DNA [16] and a single intranasal boost with a replication-competent SeV-Gag (F[+]SeV-

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Gag) [10,17], whereas macaques R003 and R006 were boosted intranasally with a recombinant SeV expressing HIV-1 Tat (F[+]SeV-Tat) after the DNA vaccinations [18]. Animals were challenged intravenously with SHIV89.6PD approximately 3 months after the SeV boost. Finally, these macaques received the second SeV vaccination; macaques R011 and R012 were vaccinated with replication-defective F-deleted SeV-Gag (F[−]SeV-Gag) [19] at week 191 and euthanized at week 196 after the first F(+)SeV-Gag vaccination, whereas R003 and R006 were vaccinated with F(+)SeV-Gag at week 68 and euthanized at week 69 (R003) or 70 (R006) after the first F(+)SeV-Tat vaccination. These macaques were maintained in accordance with the Guideline for Laboratory animals of National Institute of Infectious Diseases and National Institute of Biomedical Innovation.

Measurement of plasma anti-SeV IgG levels. The plasma anti-SeV immunoglobulin G (IgG) levels were measured by an enzyme-linked immunosorbent assay (ELISA) (Denka Seiken, Tokyo, Japan) using whole inactivated SeV (HVJ Z strain) particles and a peroxidase-conjugated anti-monkey IgG antibody [20].

SeV neutralization assay. A recombinant SeV expressing EGFP (SeV-EGFP) was obtained as described before [17]. Virus titer was determined by infecting LLCMK2 cells and counting the number

Table 1
SeV vaccination and Gag-specific CD8⁺ T-cell responses

Macaques	SeV vaccination[a]		Gag-specific CD8 T-cell frequencies[b]	
	1st	2nd	Just before the 2nd SeV	1 week after the 2nd SeV
	At week 0	At week 191	At week 191	At week 192
R011	F(+)SeV-Gag	F(−)SeV-Gag	5.0×10^2	1.1×10^3
R012	F(+)SeV-Gag	F(−)SeV-Gag	1.6×10^2	1.3×10^3
	At week 0	At week 68	At week 68	At week 69
R003	F(+)SeV-Tat	F(+)SeV-Gag	5.0×10^2	2.2×10^3
R006	F(+)SeV-Tat	F(+)SeV-Gag	2.5×10^2	1.2×10^3

[a] Macaques R011 and R012 received the second SeV vaccination at week 191 after the first SeV vaccination, and R003 and R006 at week 68.
[b] Gag-specific CD8⁺ T-cell frequencies per million peripheral blood mononuclear cells (PBMCs) reported previously [15] are shown.

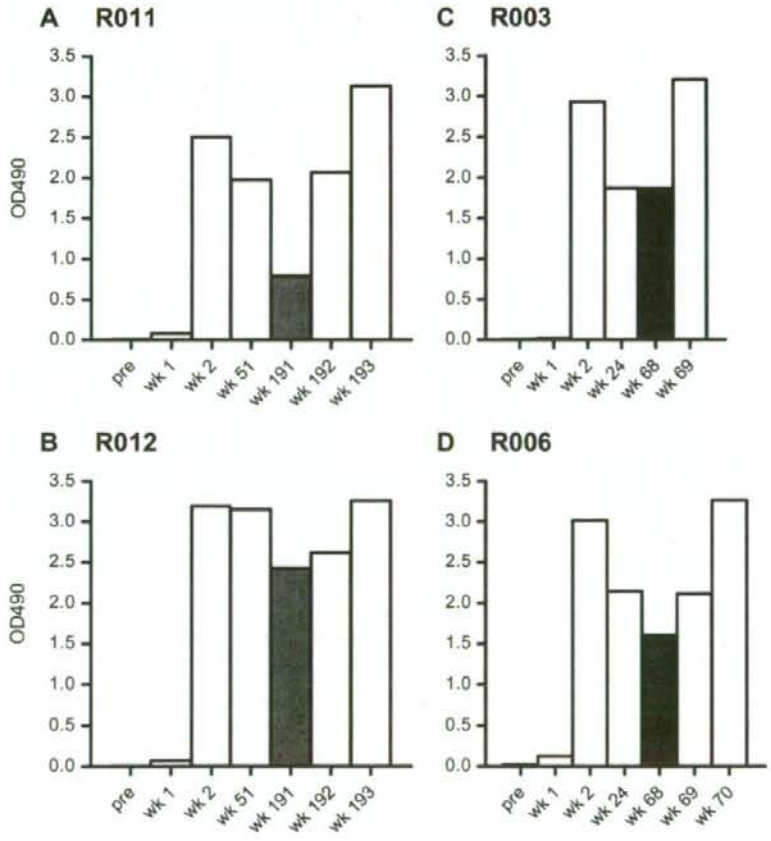

Fig. 1. SeV-specific IgG levels in plasma. Plasma samples obtained from macaques R011 (A), R012 (B), R003 (C), and R006 (D) before the initial DNA vaccination (pre) and at several time points after the first SeV vaccination were diluted by 1/5000 and subjected to ELISA assay. OD490, optical density at 490 nm.

of GFP-expressing cells. To assess the infectivity of SeV-EGFP on LLCMK2 cells, cells were plated at a density of 3.0×10^4 cells per well in 96-well plates, incubated overnight, and infected with serial twofold dilutions of SeV-EGFP. One day after the infection, cells were harvested by using 0.05% trypsin with 0.02% EDTA and subjected to flow-cytometric analysis for detection of EGFP-positive cells.

To measure SeV-specific neutralizing titers on LLCMK2 cells, cells were plated at a density of 3.0×10^4 cells per well in 96-well plates, incubated overnight, and infected with the mixture of SeV-EGFP and diluted plasma. For preparation of the mixture, 25 μl of virus solutions containing 8.3×10^4 cell infectious units (CIU) of SeV-EGFP were incubated with equal volume of serial twofold dilutions of heat-inactivated plasma samples at 37 °C for 1 h. One day after the infection, cells were harvested and subjected to flow-cytometric analysis for detection of EGFP-positive cells. Percent neutralizing activity was calculated by subtracting the percentage of the EGFP-positive cell number in the culture with plasma samples per that without plasma from 100%.

Results and discussion

In the present study, we examined SeV-specific antibody responses in plasma samples of four rhesus macaques that had received SeV vector vaccination twice as described previously [15]. At the second vaccination, macaques R011 and R012 received F(−)SeV-Gag at week 191 after the first vaccination, while macaques R003 and R006 received F(+)SeV-Gag at week 68. In all these macaques, Gag-specific $CD8^+$ T-cell responses were augmented after the second vaccination (Table 1).

We first measured plasma anti-SeV IgG levels in these macaques (Fig. 1). All the macaques showed efficient induction of SeV-specific antibody responses 2 weeks after the first SeV vaccination. High levels of anti-SeV IgG were maintained until the second vaccination and enhancement of the SeV-specific antibody responses were observed after that. This enhancement appeared 1 week after the second SeV vaccination, indicating rapid secondary responses.

Next, we established a method for measurement of SeV-specific neutralizing titers on LLCMK2 cells by using SeV-EGFP. Titration of SeV-EGFP on LLCMK2 cells roughly exhibited a proportional relationship between viral titers and GFP-positive cell frequencies in the m.o.i. (multiplicity of infection) range from 0.2 (6.3×10^3 CIU/well) to 3.3 (1.0×10^5 CIU/well) (Fig. 1A). Then, in the neutralizing assay, 25 μl of virus solution containing 8.3×10^4 CIU of SeV-EGFP was mixed with 25 μl of diluted plasma and added into each well (m.o.i. = 2.8), and GFP-positive cell frequencies were measured.

Incubation of SeV-EGFP with serially diluted pre-immune plasma samples did not affect SeV infectivity and showed similar levels (approximately 75%) of GFP-positive cell frequencies (Fig. 2B). In contrast, neutralization of SeV infection was observed by incubation with plasma samples obtained at week 2 after the first SeV vaccination (Fig. 2B).

We then measured SeV-specific neutralizing titers just before the second SeV vaccination. We determined the end-point plasma titers required for 10-fold reduction of GFP-positive cell frequencies compared to the negative control without plasma (90% neutralization) (Fig. 3). Analyses revealed efficient SeV-specific neutralizing titers in plasma just before the second SeV vaccination in all four macaques. The 90% neutralization titers were 1:100 in macaques R003 and R006, 1:200 in macaque R011, and 1:800 in macaque R012. Higher neutralizing titer in macaque R012 compared to other three was compatible with their anti-SeV IgG levels. The second SeV vaccination resulted in increases in SeV-specific neutralizing titers by fourfold or more.

In macaques R011 and R012, Gag-specific $CD8^+$ T-cell frequencies (cells/million PBMCs) after the first SeV-Gag vaccination were

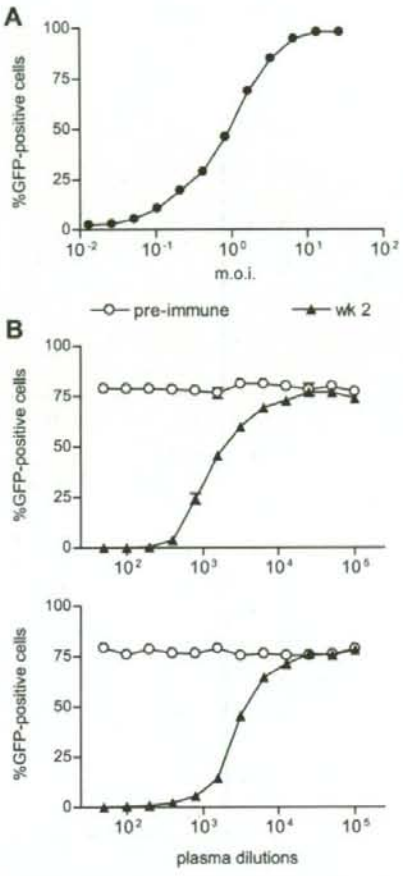

Fig. 2. GFP-positive cell frequencies after SeV-EGFP infection. (A) GFP-positive cell frequencies after infection with diluted SeV-EGFP. LLCMK2 cells were infected with serial twofold dilutions of SeV-EGFP. In case of m.o.i. of 3.3, 3.0×10^4 cells were plated per well in the 96-well plate, incubated overnight, and infected with 1.0×10^5 CIU of SeV-EGFP. The mean values obtained by duplicate experiments are shown. (B) GFP-positive cell frequencies after infection with the mixture of SeV-EGFP and diluted plasma samples. SeV-EGFP (8.3×10^4 CIU) was incubated with serially diluted plasma obtained pre-vaccination (pre-immune, open circles) or at week 2 after the first SeV immunization (closed triangles) from macaques R011 (upper panel) and R003 (lower panel) and added into LLCMK2 cells plated at 3.0×10^4 cells per well. The mean values obtained by duplicate experiments are shown.

approximately 1.0×10^3 [10] and those after the second SeV-Gag vaccination were just above 1.0×10^3 (Table 1) [15]. Granted that the priming conditions and the utilized vectors were different between the first and the second SeV vaccination, these results indicate efficient Gag-specific $CD8^+$ T-cell induction even by the second SeV-Gag vaccination. Thus, our finding of SeV-specific neutralizing antibody responses just before the second SeV-Gag vaccination in the present study indicates the potential of recombinant SeV vectors to induce antigen-specific T-cell responses even in the presence of SeV-specific neutralizing antibodies, suggesting an important implication for development of an effective AIDS vaccine using viral vectors.

Because of the cross-reactivity of anti-SeV antibodies with hPIV-1, a clinical trial phase I of wild-type SeV vaccination against hPIV-1 infection has been performed [21]. However, the potential

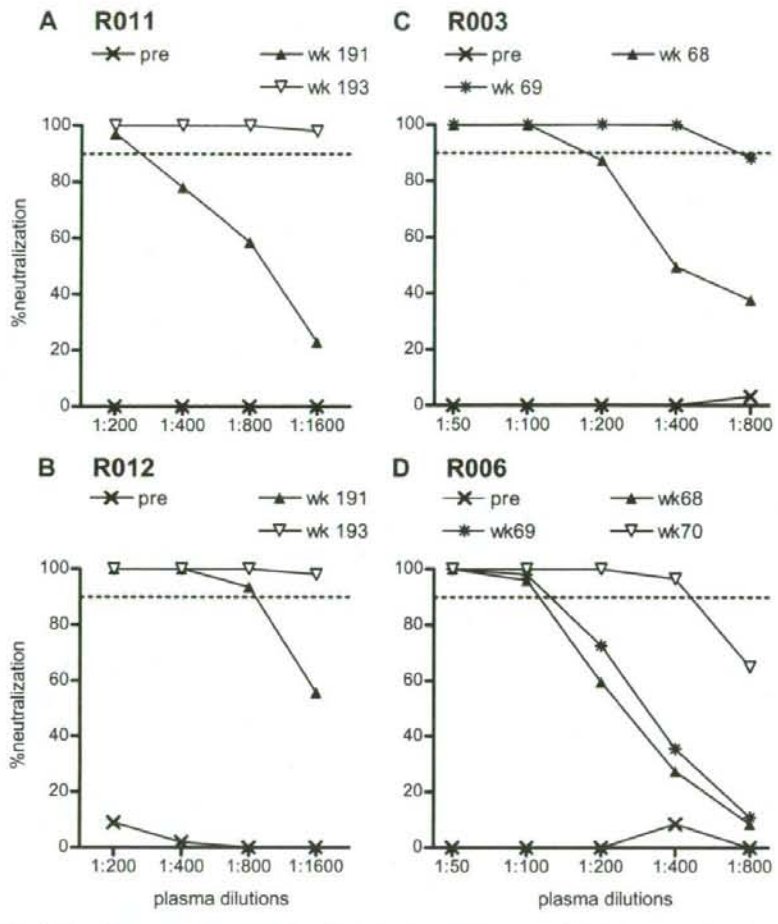

Fig. 3. SeV-specific neutralizing titers in plasma. We examined neutralizing titers in plasma samples obtained before the initial DNA vaccination (pre), just before the second SeV vaccination (at week 191 in R011 and R012 and at week 68 in R003 and R006), and after that in macaques R011 (A), R012 (B), R003 (C), and R006 (D). The mean values obtained by duplicate experiments are shown.

of SeV vaccination to induce hPIV-1-specific neutralizing antibody responses has not precisely evaluated. Conversely, anti-hPIV-1 antibodies may cross-react with SeVs, but how much extent these can neutralize SeVs remains unclear. While we do not know the frequency of anti-hPIV-1 antibody-positive individuals, the present study strongly suggests the feasibility of efficiently inducing antigen-specific T-cell responses by SeV vaccination even in the host with pre-existing anti-hPIV-1 antibodies. Precise evaluation of relationship between pre-existing SeV-specific neutralizing titers and efficiency of antigen-specific T-cell induction would contribute to estimation of SeV vector vaccine efficacy in anti-hPIV-1 antibody-positive individuals.

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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

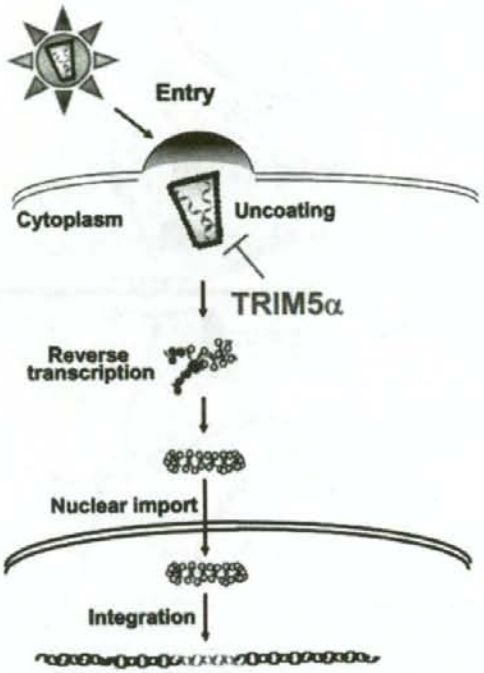

Fig. 1. A schema for TRIM5α-mediated restriction of HIV-1 replication in OWM cells. Recognition of HIV-1 CA by TRIM5α results in restriction of HIV-1 replication at the step after viral entry into the cytoplasm.

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

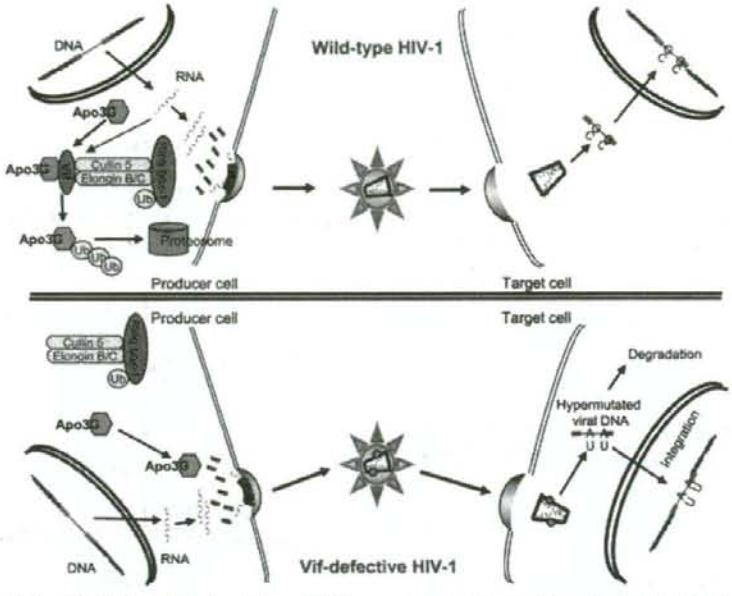

Fig. 2. A putative model for APOBEC3G-mediated restriction of HIV-1 replication and Vif-mediated recovery from the restriction. In the wild-type HIV-1 replication (upper panel), Vif connects hApo3G to an E3 ubiquitin ligase complex including Elongin B/C, Cullin5, and Ring-box-1 to induce polyubiquitylation 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

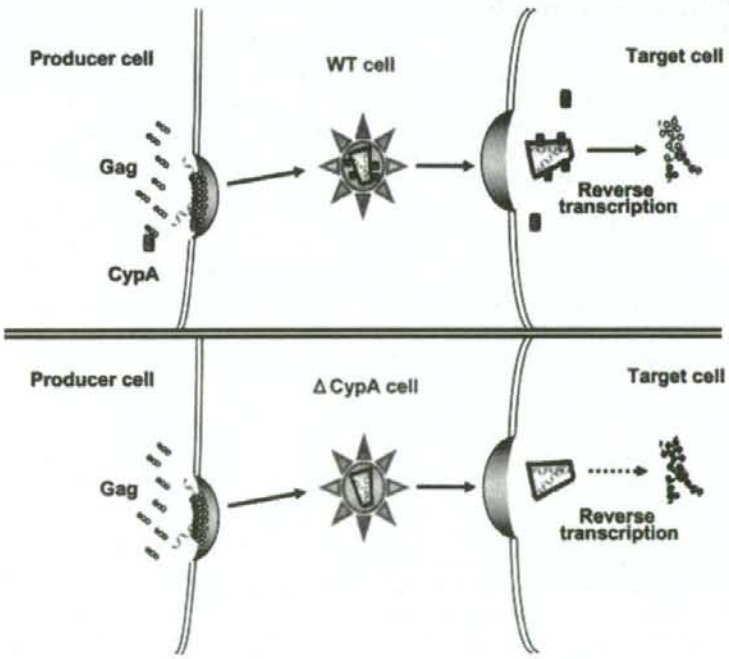

Fig. 3. A putative mechanism for CypA-mediated enhancement of HIV-1 replication. CypA is required for efficient reverse transcription (upper panel), and HIV-1 infection in CypA-deficient human cells shows inefficient reverse transcription (lower panel).

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.

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REVIEW

Anti-HIV adaptive immunity: determinants for viral persistence

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SUMMARY

The immense difficulty in primary control of human immunodeficiency virus (HIV) and simian immunodeficiency virus (SIV) infection by adaptive immune responses has been a topic of exceptional importance. $CD8^+$ cytotoxic T lymphocytes (CTLs) do play a central role in primary resolution of viremia, but their potency in viral control is generally constrained in the natural courses of HIV/SIV infections. The overall repertoire of CTLs is dependent on both the host and the virus genetic polymorphisms, and the potency of each individual CTL is affected by immunological and virological determinants. HIV/SIV infections lack early appearance of neutralising antibodies (NAbs), and our recent finding has suggested a possibility of their absence contributing to diminished virus-specific $CD4^+$ T-cell responses leading to failure in primary viral control. Extrapolations from studies in macaque models of SIV infection and analyses of the cohorts of HIV control in humans have to date delineated the numerous requirements for attainment of viral control. Understanding of the individual components of adaptive immune responses and their optimal concert required for HIV/SIV control would contribute to development of an effective AIDS vaccine. Here, we discuss current insights into CTLs and NAbs, and speculate their possible protective mechanism against establishment of persistent HIV/SIV infection. Copyright © 2008 John Wiley & Sons, Ltd.

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ACUTE MEMORY $CD4^+$ T-CELL DEPLETION BY HIV INFECTION

The kinetics of human immunodeficiency virus (HIV) infection, especially in the acute phase, has been a long-pursued question. The first turning point came with identification of chemokine receptors including CCR5 as the coreceptor for viral entry into the cells [1–4], contributing to determination of the primary viral target cell population. CCR5-tropic viruses were shown to play a central role in viral transmission [5–7], and studies using macaques infected with CCR5-tropic simian immunodeficiency virus (SIV) revealed predominant viral infection of activated $CD4^+$ T lymphocytes in the gut in the early phase [8,9]. Subsequent key studies showed acute depletion of memory $CD4^+$ T lymphocytes that were enriched with CCR5 expression [10,11], starting at the gut lamina propria around week 1 and then systemically around week 2 post-infection [12–14]. This CCR5-tropic depletion can be an obstacle to *de novo* induction of potent virus-specific immune responses, which may contribute to failure in primary viral control [15].

In agreement with these findings, trials of prophylactic AIDS vaccines eliciting $CD8^+$ cytotoxic T lymphocyte (CTL) responses in macaques against chimeric CXCR4-tropic simian-human immunodeficiency viruses (SHIVs) and CCR5-tropic SIVs have revealed an immense difference in attainability of viral control between the two. In the former model of CXCR4-tropic SHIV infection that shows aggressive natural infection courses with massive acute depletion of naïve

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Abbreviations used

HIV, human immunodeficiency virus; SIV, simian immunodeficiency virus; SHIV, simian-human immunodeficiency virus; CTL, cytotoxic T lymphocyte; HLA, human leukocyte antigen; MHC, major histocompatibility complex; MHC-I, MHC class I; NAb, neutralising antibody; hu-PBL-SCID mice, human peripheral blood leukocyte-reconstituted severe combined immunodeficiency mice; DC, dendritic cell.