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Fig. 1

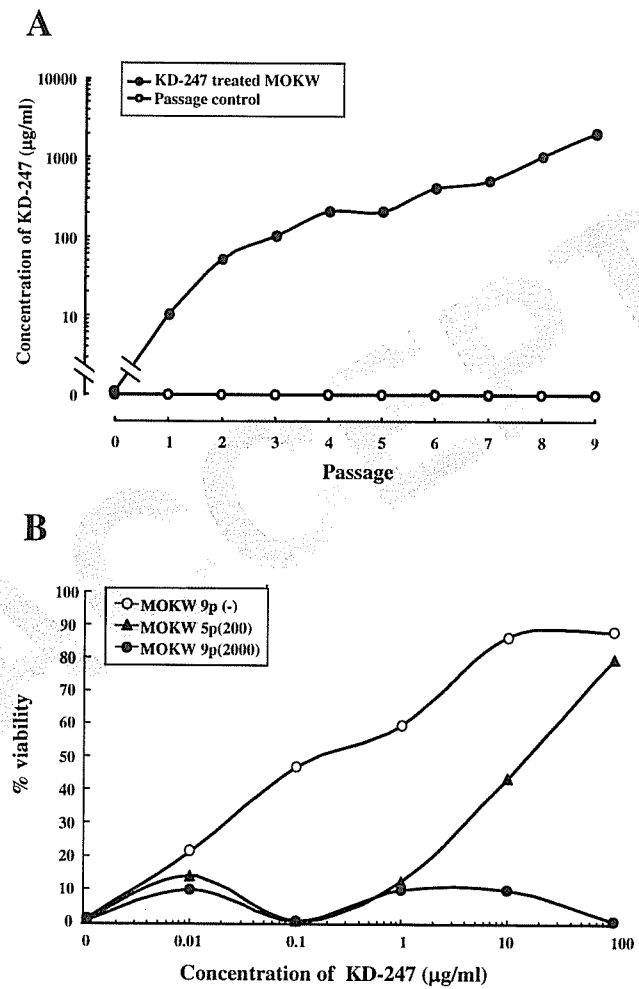
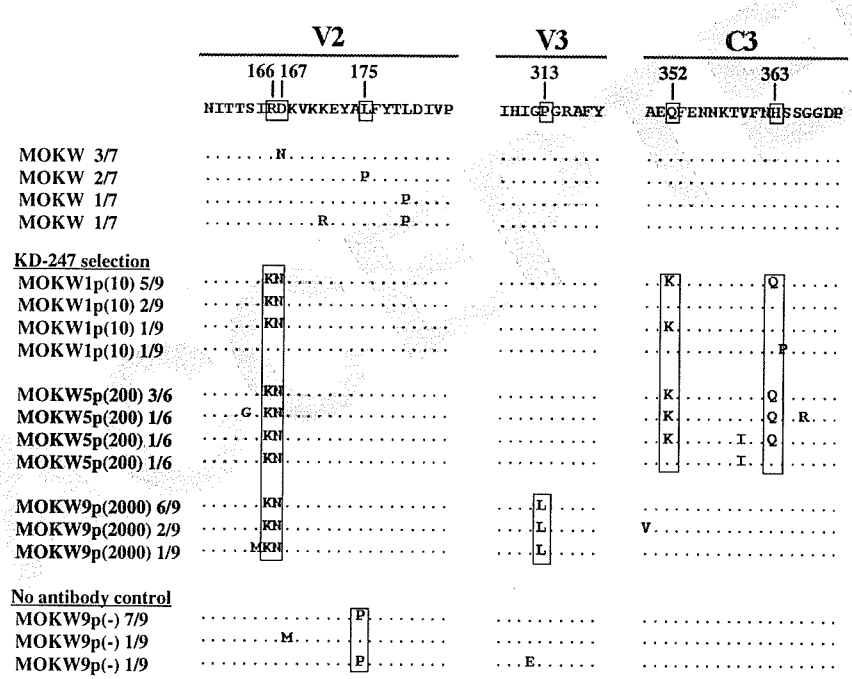


Fig. 2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V3
C3
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175
313
352
363
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MOKW 3/7
MOKW 2/7
MOKW 1/7
MOKW 1/7
KD-247 selection
MOKW1p(10) 5/9
MOKW1p(10) 2/9
MOKW1p(10) 1/9
MOKW1p(10) 1/9
MOKW5p(200) 3/6
MOKW5p(200) 1/6
MOKW5p(200) 1/6
MOKW5p(200) 1/6
MOKW9p(2000) 6/9
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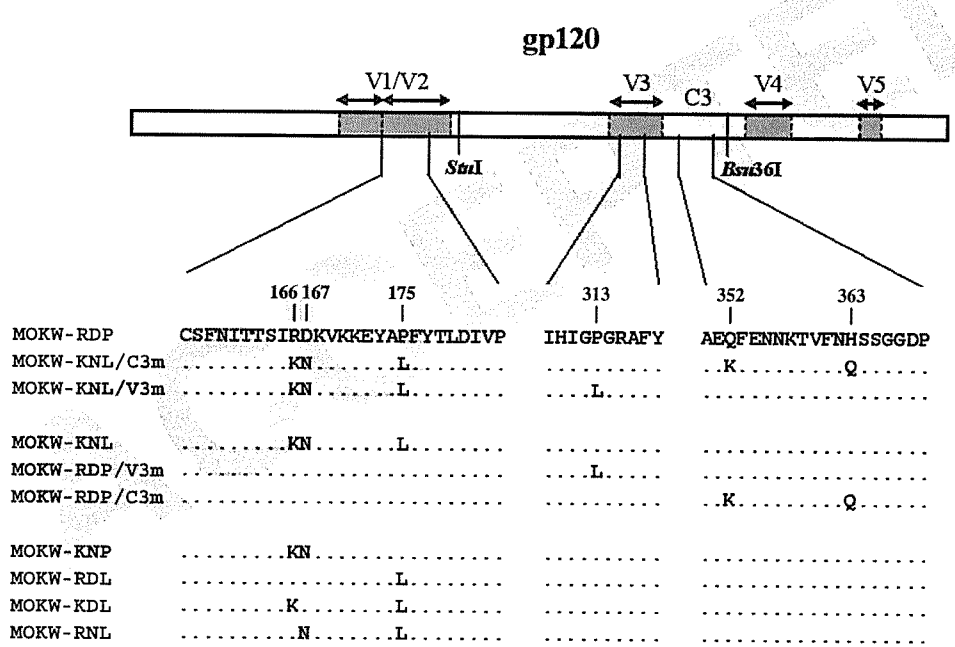
MOKW9p(-) 1/9
MOKW9p(-) 1/9

Fig. 3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V3
C3
V4
V5
StuI
Bsu36I
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175
313
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MOKW-KNL/C3m .....KN.....L..... ....K.....Q.....
MOKW-KNL/V3m .....KN.....L..... ....L..... .....
MOKW-KNL .....KN.....L..... ....
MOKW-RDP/V3m ....L..... .....
MOKW-RDP/C3m ....K.....Q.....
MOKW-KNP .....KN..... ....
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Fig. 4

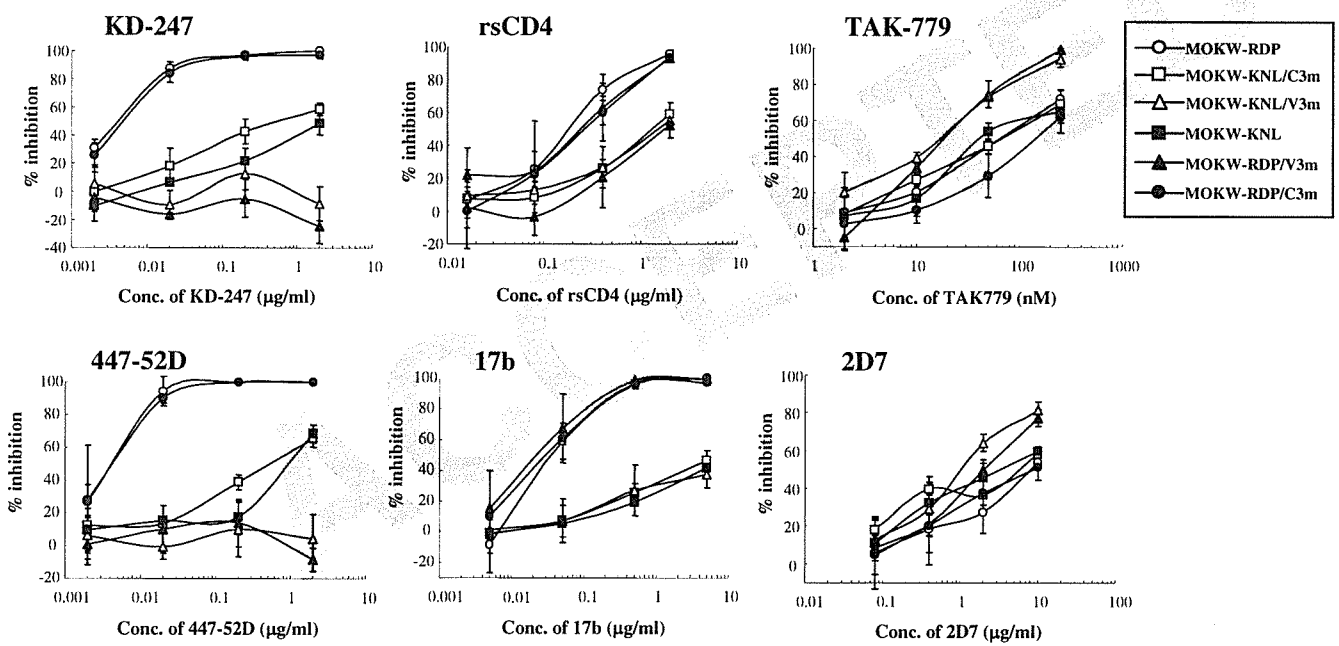
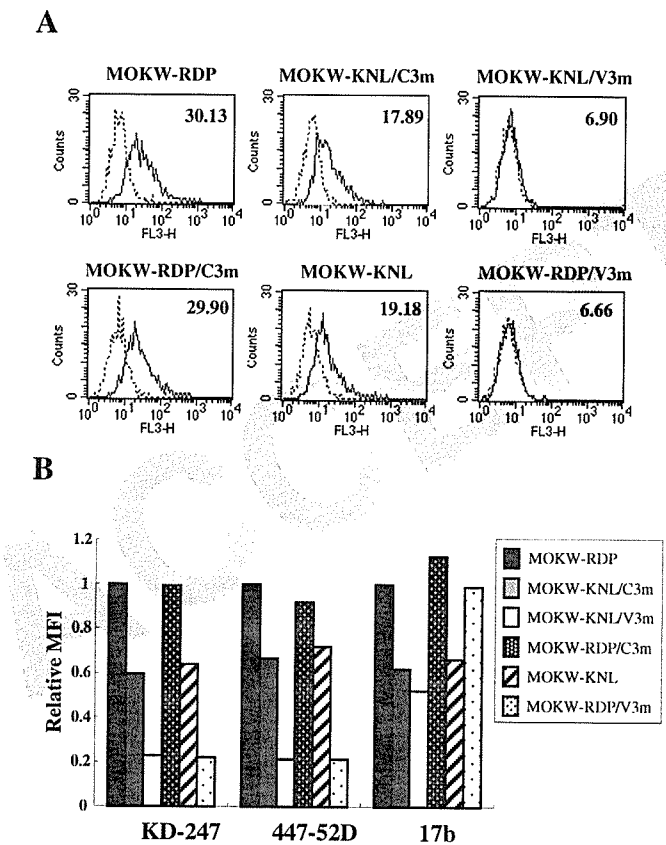

KD-247
% inhibition
Conc. of KD-247 (μg/ml)
rsCD4
Conc. of rsCD4 (μg/ml)
TAK-779
Conc. of TAK779 (nM)
MOKW-RDP
MOKW-KNL/C3m
MOKW-KNL/V3m
MOKW-KNL
MOKW-RDP/V3m
MOKW-RDP/C3m
447-52D
Conc. of 447-52D (μg/ml)
17b
Conc. of 17b (μg/ml)
2D7
Conc. of 2D7 (μg/ml)

Fig. 5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MOKW-KNL/V3m
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MOKW-RDP/C3m
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MOKW-KNL
19.18
MOKW-RDP/V3m
6.66
Counts
FL3-H
B
Relative MFI
1.2
1
0.8
0.6
0.4
0.2
0
KD-247
447-52D
17b
MOKW-RDP
MOKW-KNL/C3m
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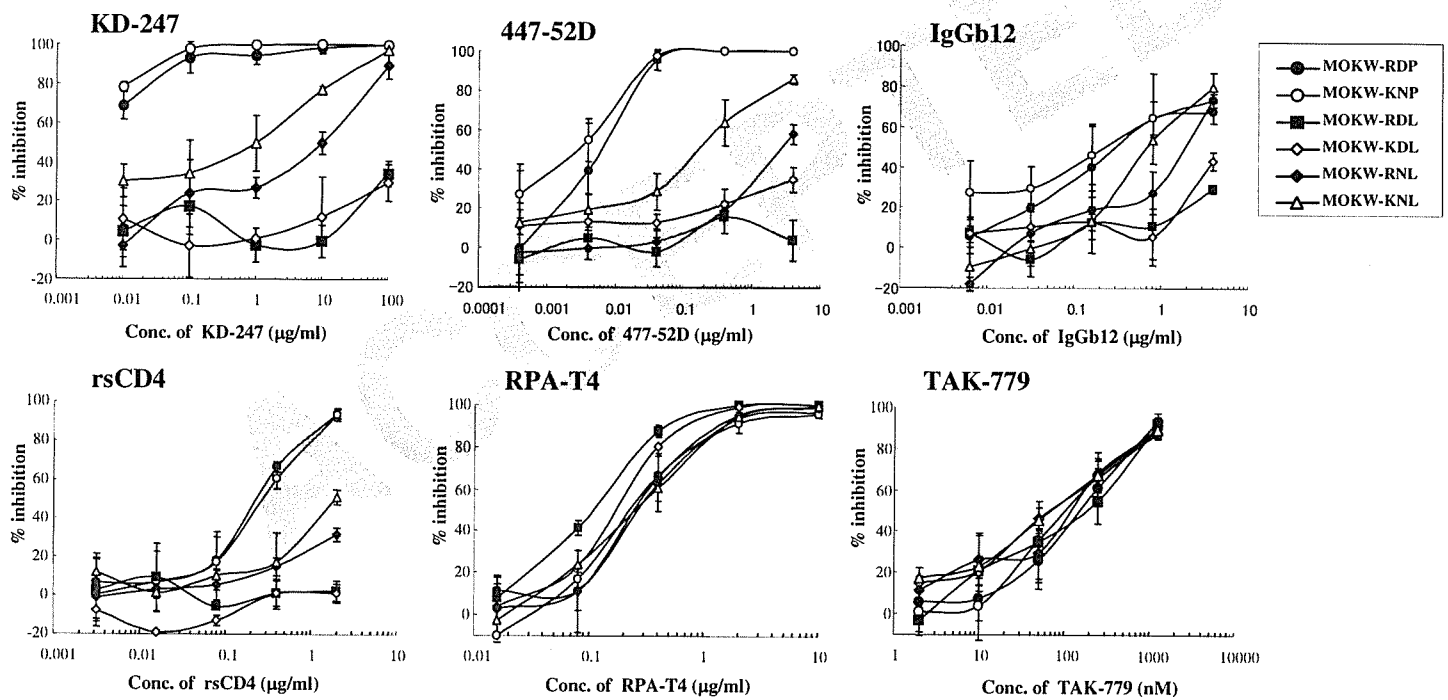
MOKW-RDP/C3m
MOKW-KNL
MOKW-RDP/V3m

Fig. 6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IgGb12
rsCD4
RPA-T4
TAK-779
% inhibition
Conc. of KD-247 (μg/ml)
Conc. of 477-52D (μg/ml)
Conc. of IgGb12 (μg/ml)
Conc. of rsCD4 (μg/ml)
Conc. of RPA-T4 (μg/ml)
Conc. of TAK-779 (nM)
MOKW-RDP
MOKW-KNP
MOKW-RDL
MOKW-KDL
MOKW-RNL
MOKW-KNL

Fig. 7

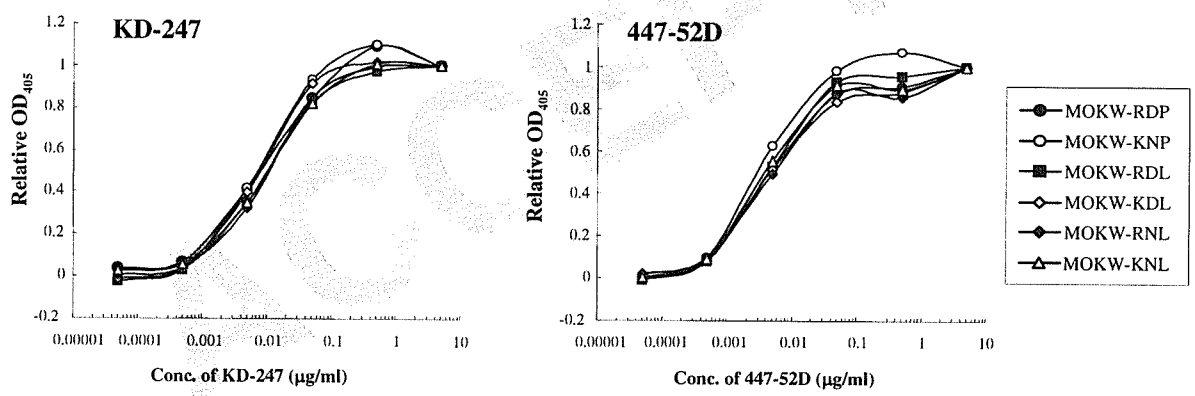
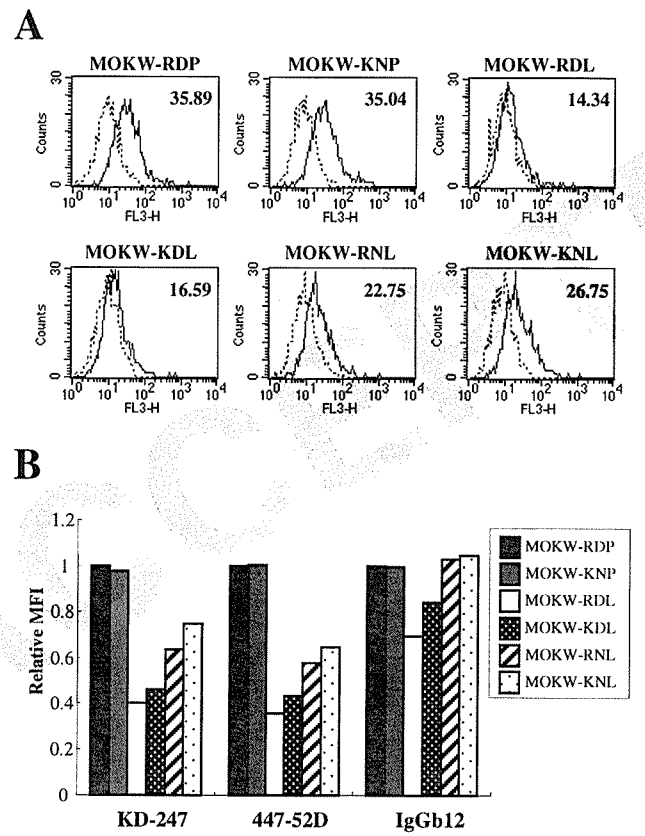


Fig. 8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MOKW-KNP
35.04
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14.34
MOKW-KDL
16.59
MOKW-RNL
22.75
MOKW-KNL
26.75
Counts
FL3-H
B
Relative MFI
1.2
1
0.8
0.6
0.4
0.2
0
KD-247
447-52D
IgGb12
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MOKW-RDL
MOKW-KDL
MOKW-RNL
MOKW-KNL

Fig. 9

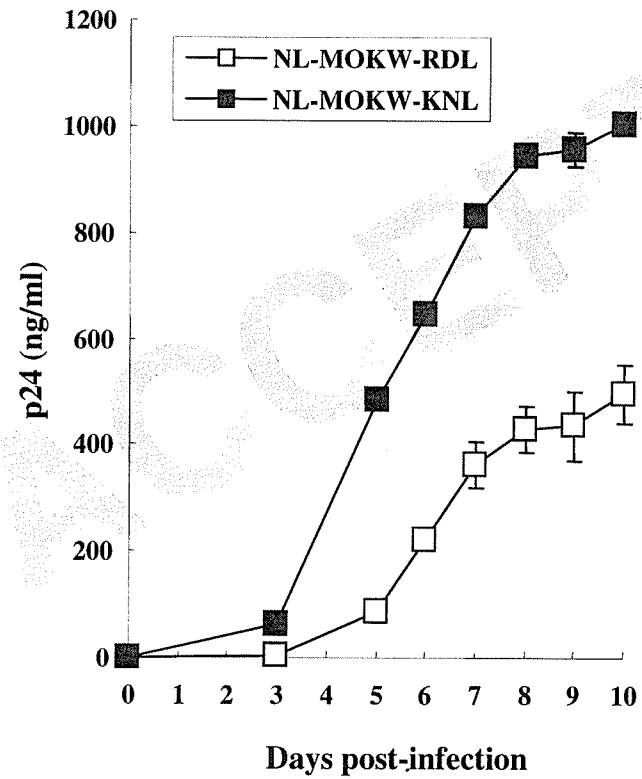

Table 1. Anti-HIV-1 activities of various MAbs and inhibitors toward MOKW pseudoviruses

Class	Compound		IC_{50}[a]					
			MOKW-RDP	MOKW-KNL/C3m	MOKW-KNL/V3m	MOKW-RDP/C3m	MOKW-KNL	MOKW-RDP/V3m
V3 MAbs	**KD-247**	(μg/ml)	0.004 (x1)[b]	0.5 (x125)	>100 (>x25000)	0.005 (x1.3)	2 (x500)	>100 (>x25000)
	447-52D	(μg/ml)	0.004 (x1)	0.5 (x125)	>2 (>x500)	0.004 (x1)	0.8 (x200)	>2 (>x500)
CD4-induced MAb	**17b**	(μg/ml)	0.035 (x1)	>5 (>x143)	>5 (>x143)	0.03 (x0.86)	>5 (>x143)	0.02 (x0.57)
CD4	**rsCD4**	(μg/ml)	0.18 (x1)	1.3 (x7.22)	1.5 (x8.33)	0.24 (x1.33)	1.8 (x10)	0.24 (x1.33)
CCR5 MAb	**2D7**	(μg/ml)	8 (x1)	6.8 (x0.85)	1 (x0.13)	8 (x1)	3.2 (x0.4)	2 (x0.25)
CCR5 small molecule	**TAK-779**	(nM)	63 (x1)	63 (x1)	18 (x0.29)	140 (x2.22)	65 (x1)	18 (x0.29)
CD4 MAb	**RPA-T4**	(μg/ml)	0.4 (x1)	0.26 (x0.65)	0.22 (x0.55)	0.5 (x1.25)	0.22 (x0.55)	0.44 (x1.1)

[a]GHOST-hi5 cells were exposed to 100 $TCID_{50}$ of each MOKW pseudovirus and then cultured in the presence of various concentrations of MAb or inhibitors. The IC_{50} values were determined using the luciferase reporter assay on day 2 of culture. All assays were conducted in triplicate. The values shown are representative of two or three separate experiments.

[b]The values in parentheses are relative IC_{50} values (relative to the IC_{50} value of each compound for MOKW-RDP).

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A Single-Nucleotide Synonymous Mutation in the *gag* Gene Controlling Human Immunodeficiency Virus Type 1 Virion Production▽

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Viral factors as well as host ones play major roles in the disease progression of human immunodeficiency virus type 1 (HIV-1) infection. We have examined cytotoxic T-lymphocyte activity and HIV-1 DNA PCR results of 312 high-risk seronegative drug users in northern Thailand and identified four seronegative cases positive for both assays. Furthermore, we have identified a synonymous mutation in nucleotide position 75 of the *gag* p17 gene (A426G) of HIV-1 that belongs to the CRF01_AE virus circulating in Thailand. The replication-competent HIV-1 clone containing the A426G mutation demonstrated a dramatic reduction of virion production and perturbation of viral morphogenesis without affecting viral protein synthesis in cells.

Indirect evidence of abortive human immunodeficiency virus type 1 (HIV-1) infection in cases of highly exposed persistently seronegative individuals and transient seroconversion have been reported (2, 3, 5, 8, 10, 13, 16, 19–23) wherein host and viral factors are considered to play major roles. The host factors were deemed responsible for the natural resistance to HIV-1 infection; however, the viral factors responsible for the abortive HIV infection process have not been fully understood. For example, Zhu et al. (26) reported remarkable genetic stability of *gag* and *env* sequences of HIV-1 proviral DNA in resting $CD4^+$ T cells obtained from highly exposed persistently seronegative individuals, indicating the involvement of viral factors. Moreover, attenuated HIV-1 isolates obtained from long-term nonprogressors (14) were reported, demonstrating the diminished transmissibility of the virus. These findings indicate that viral factors are responsible for abortive HIV-1 infection. Understanding such mechanisms would be beneficial for the development of novel therapeutic and preventive strategies against HIV-1.

In this study, we focused on the drug user (DU) cohort study conducted in northern Thailand wherein current epidemiological surveys have revealed a dramatic decrease in seroprevalence and incidence rate of HIV-1 infection in spite of unchanged behaviors among DUs (9). A total of 421 cases (HIV-1 seronegative, $n = 320$; seropositive, $n = 101$) in the DU cohort were enrolled with written informed consent from January 1999 to November 2000 and followed up (every 6 months) until November 2003 (Fig. 1A). Eight cases among 320 seronegative DUs had seroconverted, whereas the other 312 cases remained seronegative throughout the study. All enrollment samples were stored, and subsequently diagnostic and PCR tests for HIV-1 were performed in separate laboratories in Thailand to eliminate the possibility of cross-contamination. Duplicate follow-up specimens were also assayed for HIV-1 *gag* p17 by PCR test and sequencing (7, 15); a cytokine enzyme-linked immunospot assay kit (U-CyTech, The Netherlands) (10) was used to detect human gamma interferon responses by using overlapping (20 mer) whole Gag peptides designed from a CRF01_AE clinical isolate. Furthermore, *gag* p17 gene-containing fragments were cloned and sequenced. The nucleotide sequence alignment adjacent to nucleotide position 75 (+426) of the *gag* p17 gene of HIV-1 clones from each seronegative DU subject is shown in Fig. 1C. A mutant molecular clone, G6, containing an A426G mutation was constructed from a replication-competent HIV-1 molecular clone, G5, based on isolate 92TH022 (11).

293 cells were transfected with clones G5 and G6 using Fugene-6 transfection reagent (Roche Diagnostics, Basel, Switzerland) (24). Every 24 h posttransfection, culture supernatant was collected for determination of p24 antigen (HIV-1 p24 antigen enzyme-linked immunosorbent assay [ELISA]; ZeptoMetrix Co., Buffalo, NY) and HIV-1 RNA level (Am-

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