Korean Red Ginseng-intake has Definite Clinical Usefulness and causes Nef Gene Variation including High Frequency of Deletion

Young Keol Cho,[1] Hee Kyung Lee,[1] Sun Hee Ahn,[1] Hee Jung Lee,[1] Ki Yeul Nam[2]

Department of Microbiology, [1]Asan Medical Center, University of Ulsan, College of Medicine, Seoul 138-040, Korea, KT&G Central Research Institute, [2]Daejeon 305-345, Korea

Abstract

We have found many beneficial effects of the long-term intake of Korean red ginseng (KRG) in human immunodeficiency virus (HIV) type-1 infected patients, including the maintenance of CD4+ T cell count for 10 years with KRG only and the delayed development of resistance mutation to ZDV. In this study, to investigate whether KRG-intake could affect the clinical progression and *nef* gene variation, we determined 200 *nef* sequences from 70 patients. Follow-up period was 8.8±2.9 years and annual decrease in CD4+ T cell was 41±57/ul. Nested polymerase chain reaction (PCR) and direct sequencing were performed with peripheral blood mononuclear cells (PBMC) obtained at times during the study period. First, there was a significant correlation between survival duration and duration of KRG-intake (36.8±38 months)(P=0.000). There were significant correlations between the last NefProg score and CD4+ T cell count (r=-0.208, P<0.05) and annual decrease in CD4+ T cell count (r =0.346, P<0.01) in 70 patients. In addition, there were significant correlations between KRG-intake and annual decrease (r=-0.323, P <0.01), and the CD4+ T cell count itself (r=0.229, p<0.05). Furthermore, there was also a mild significance between the NefProg score and the duration of KRG-intake in only SP and RP (n=30, r=-0.281, P=0.067). In addition, we detected various defects in 21 patients (30.0%), not including 5 premature stop codons. Ten (12.5%) patients showed repeated deletion of an amino acid. Four of 10 patients were gross deletions and they were treated with KRG for more than 20 months. The number of patients with repeated gross deletions was significantly higher in the order of slow progressors (18%), typical progressors (3%), and rapid progressors (0%) (P<0.05). We also observed that long-term intake of KRG might make the change from A or D to T at position 54 and decrease NefProg score. Taken together, our results show clear evidence that the long-term intake of KRG has effects on *nef* gene variation as well as definite clinical usefulness.

Introduction

Less than 5% of human immunodeficiency virus type 1 (HIV-1)-infected individuals remain healthy without AIDS-defining illness for longer than 10 years in the absence of conventional antiretroviral therapy (ART) (2). Individuals who have maintained CD4+ T cell counts of more than 500/ul without ART are known as long-term nonprogressors (LTNP) (27). The *nef* gene downregulates CD4 cell surface expression and has the ability to increase viral replication in primary lymphocytes and macrophages and to enhance virion infectivity. *Nef* can also downmodulate the cell surface expression of MHC-class I molecules, an effect found to protect infected cells from killing by cytotoxic T cells. Finally, *Nef* can alter T-cell signalling pathways (15). Taken together, *nef* plays an important role in disease progression in HIV-1 infected individuals (19) similar to its role in SIV in terms of pathogenic importance in SIV and a virulence factor (23). The presence of a defective *nef* gene in LTNP have been published by several reports (9, 20, 22, 27, 29). However, Huang et al (17) found no gross defects or sequence abnormalities in *nef* alleles derived from 10 HIV-infected LTNPs. The factors involved in long-term nonprogression have been the subject of intense investigations as they may provide a clue for understanding HIV infection.

Several factors associated with slow progression have been identified as follows: polymorphism of coreceptor (32 CCR5 and CCR2b)(10, 31), HLA alleles (28), and viral phenotypes of syncytium inducing (SI) and NSI (11).

Although several defects in the viral genome have been reported, intensive research has been focused on the *nef* gene. Since the first report on the defective *nef* in 8 Australian hemophiliacs in 1995 (9), there have been 3 other reports on the grossly *nef*-defective HIV-1 strains (13, 14, 29). However, some of those individuals with defective *nef* genes have shown a decrease in CD4+ T cell count and increase in viral load after the first report. Although we have previously reported on a phylogenetic analysis on *nef* sequences in 46 patients (18), there has been no report on *nef* gene variation according to the progression and clinical state in HIV-infected Korean patients.

We have, however, tried Korean red ginseng (KRG) for HIV-infected individuals since November 1991, and have encountered many individuals with nonprogression or slow progression (3-8). In regard to our data on the effects of KRG in HIV-infected individuals, surprisingly, we have observed various beneficial effects of KRG-intake (5.4 mg daily) in these individuals including increases in the CD4+ T cell, CD8 + T cell counts and body weight, and a decrease in

soluble CD8 antigen (sCD8) in serum (4). Thereafter, the study was done consecutively for the same target population, although the number of patients was increased and there were some interruptions. Furthermore, our data showed that intra-patient amino acid variation between clones in the C2/V3 region of HIV-1 from these patients was inversely correlated with the duration of KRG-intake (5). In patients treated with ZDV and KRG combination therapy, the development of resistance to ZDV was significantly delayed, along with the maintenance of CD4+ T cell counts (8). Among the patients who have been involved in the KRG trial, some have maintained CD4+ T cell counts for 10 years with KRG-intake only.

Therefore, in this study, to investigate whether the *nef* gene could be affected by long-term intake of KRG and whether there is a correlation between slow progression and KRG-intake, we performed nested PCR and direct sequencing by using denatured PBMCs or extracted DNA prepared from 70 patients. There were significant correlations between NefProg score according to *nef* gene variation and CD4+ T cell count ($r=-0.208$, $P<0.05$), and the plasma RNA copy ($r=0.353$, $P<0.05$). Our results suggest that long-term intake of KRG can delay the progression to AIDS by slowing *nef* gene variation, whereas nucleoside reverse transcriptase inhibitors (NRTI) and even HAART did not significantly affect the progression. Our data also show that in the long run HAART has a definite limitation because it could not revert the direction of *nef* gene variation.

Materials and Method

Study populations

Many LTNPs had progressed to AIDS after reports on LTNPs (14, 26). Strictly speaking, all HIV-1-infected patients have progressed. So we classified 70 patients into slow progressors (SP), typical progressors (TP), and rapid progressor (RP) according to their annual decrease in CD4+ T cell count<20/ul, 20-60/ul, and >60/ul, respectively. In case the patient was being treated with HAART, we considered the end point to calculate annual decrease was just before HAART was started. Table 1 summarizes the detailed description about year of diagnosis, and duration of treatment with NRTI and KRG in each patient.

Amplication of nef sequences

Crude cell lysates from peripheral blood mononuclear cells (PBMCs) were used for direct DNA sequencing. In case of failure by this method, genomic DNA was extracted by standard

Table 1. Characteristics on the last nef sequences in 70 Korean HIV-1 Patients Treated with KRG

Patient Code	Risk factor	Date of Dx.	Date of Sampling	CD4+ T cell	Annual fall(/ul)	Months with RTI+H	Months with KRG	Nef Prog	No. seq	Defect in nef gene and subtype
Slow progressors (n=17)										
90-01	Homosexual	01.1990	01.2002	216	16	0	42	-3	2	
90-05	Homosexual	02.1990	08.2002	233	4	0	124	-4	8	
90-18	Homosexual	05.1990	04.2002	511	15	0	87	-3	8	ΔG (2)+2aa(4)
90-50	Homosexual	12.1990	06.2002	762	14	0	88	-4	5	
91-20	Hetero	05.1991	08.2002	507	+10	0	128	-3	6	
92-13	Hemophilia	02.1992	02.2001	321	3	0	36	-4	3	Pol
89-05	Homosexual	04.1989	08.1996	417	16	0	0	-2	1	
92-shj	Hemophilia	03.1992	10.1999	423	+39	0	3	-3	4	
92-mk	Hemophilia	02.1992	12.2001	722	+29	0	4	-3	2	
92-23	Homosexual	03.1991	06.1996	1082	+71	0	5	-4	1	
92-75	Hemophilia	12.1992	03.2001	229	10	0	7	-5	4	Dlc (4)+ ΔG (1)
92-16	Hemophilia	02.1992	06.2002	291	13	0	115	-3	3	
92-76	Homosexual	12.1992	11.1996	314	+18	0	43	-3	2	
93-01	Homosexual	12.1992	08.2002	669	+73	0	97	3	4	D5aa(4)+Pre, Sub D
93-04	Homosexual	01.1993	07.2002	468	1	0	21	-5	3	
93-60	HIV spouse	10.1993	11.2001	487	13	0	21	-5	13	ΔG (3), 2Pre+I"
96-51	Homosexual	05.1996	04.2002	766	+7	0	18	-5	2	
Rapid progressors (n=17)										
87-08	Homosexual	08.1987	05.1995	41	91	41	14	4	1	Dlaa[b]
88-01	Prostitute	06.1988	02.1996	42	129	0	15	4	1	Dlc
88-12	Prostitute	06.1988	07.1997	1	107	24	9	5	1	Pol [d]
89-07	Homosexual	05.1989	12.1994	6	184	9	7	-1	1	
89-10	Overseas	05.1989	09.2000	297	83	0	12	-3	1	
89-12	Homosexual	06.1989	12.2000	53	88	0	0	-1	1	
89-15	Overseas	07.1989	01.2002	293*	77	36+60	101	3	2	Pol
89-31	Overseas	11.1989	06.1993	366	71	0	0	5	2	D3aa (2)
90-12	Homosexual	03.1990	03.1999	8	61	12	6	-3	3	
90-36	Overseas	09.1990	06.1993	547	103	0	4	4	2	PxxP, Pre, Sub G
91-16	Hemophilia	04.1991	05.1996	296	82	0	2	-3	4	
92-01	Homosexual	01.1992	05.1997	4	170	0	0	0	1	
92-05	Hemophilia	02.1992	12.2001	590*	157	59	9	-1	3	ΔG (1), Pol
92-06	Hemophilia	02.1992	08.1997	2	112	4	4	-1	4	ΔG (1), Pol, PxxP
92-32	Homosexual	05.1992	09.1996	4	95	30	18	-2	1	
92-42	Homosexual	07.1992	11.1996	28	75	4	3	-1	3	
00-179	Sex worker	12.2000	04.2002	186	296	0	3	4	3	CRF02
Typical progressors (n=36)										
91-14	Hemophilia	04.1991	07.2002	438*	46	51+67	125	3	4	D2aa+Dlc (4),ΔG, Pol
87-03	Prostitute	04.1987	03.2000	6	32	40	32	0	2	Dlc (2)[a]
87-05	Hemophilia	06.1987	03.2000	136	40	0	72	5	7	d3aa (4), I + Pre (1)
87-09	Prostitute	12.1987	09.2001	642*	48	17+46	17	1	1	
88-01	Overseas	01.1988	04.2002	241	10	120	10	4	1	CRF01_AG
88-04	HIV spouse	02.1988	07.2001	77	8	16	6	1	2	Dlc[c], Pol UNT[e]

Table 1. Continued

Patient Code	Risk factor	Date of Dx.	Date of Sampling	CD4+ T cell	Annual fall(/ul)	Months with RTI+H	Months with KRG	Nef Prog	No. seq	Defect in nef gene and subtype
88-17	Overseas	11.1988	10.2000	322	52	0	98	2	4	Sub A1
88-22	Overseas	12.1988	12.1995	392	42	0	21	4	2	CRF01_AG
89-02	Overseas	03.1989	07.2001	71	32	18	19	2	2	Pol, CRF02, ΔG(2)
89-17	Homosexual	07.1989	01.2002	106	23	0	36	-4	3	
89-18	Hetero	08.1989	12.2000	28*	45	+21	10	-3	2	D2aa (2)
89-26	Homosexual	10.1989	03.2000	28*	49	+12	0	-1	3	ΔG (1)
90-06	Homosexual	03.1990	01.1999	331	+6	29	47	1	2	
90-14	Overseas	04.1990	12.2001	366	32	0	10	4	2	CRF02
90-22	Homosexual	06.1990	03.1997	222	37	65	69	-1	3	
90-35	Homosexual	09.1990	02.2001	61	35	0	72	4	2	PxxP (2)
90-37	HIV spouse	09.1990	11.1999	209	46	0	8	1	2	CRFcpx
90-38	Homosexual	09.1990	03.2002	187*	45	+17	35	1	3	D2aa (1), Pol
90-39	Hetero	09.1990	03.2002	63	53	0	115	-3	4	ΔG (1)
90-52	Homosexual	12.1990	02.2001	260	37	0	99	-5	4	
90-54	Overseas	12.1990	12.1995	81	10	60	38	5	2	Dlc (1), PxxP
91-05	HIV spouse	02.1991	02.2001	191	14	100	102	-3	4	Pol
91-11	Overseas	03.1991	01.2002	46	47	5	50	1	4	D4aa + Dlc (4), CRF
91-15	Hemophilia	04.1991	01.1997	252	57	0	36	-4	3	
91-22	Transfusion	06.1991	12.2001	387	15	19	0	-3	2	
91-23	Transfusion	06.1991	07.2002	121*	30	80+18	19	-1	5	PxxP (1), Pol, ΔG (2)
91-29	Transfusion	06.1991	06.2002	873	+58	126	118	-3	5	
91-32	Overseas	08.1991	04.1994	364	38	0	14	0	1	Sub A
92-08	Overseas	02.1991	11.2000	142	45	0	20	-3	1	
92-31	Homosexual	05.1992	03.2001	653	24	57	70	-3	1	
92-38	Homosexual	06.1992	02.2001	334	10	41	22	-2	2	Pre (1)
92-41	Homosexual	06.1992	11.2000	38	31	37	33	-1	2	
92-48	Homosexual	08.1992	01.1997	184	6	32	43	-2	4	Pre (1)
92-62	Homosexual	10.1992	04.2001	144	25	21	16	-3	1	Pre (1)
93-12	Hemophilia	03.1993	08.2002	343*	38	56+35	84	-1	2	Gross
95-95	Homosexual	09.1995	09.2001	430	24	11	6	-3	1	I + 3 Pre

methods from PBMC samples continuously frozen at –70°C since collection. To amplify proviral *nef* sequences, a nested polymerase chain reaction (PCR) was employed with the following primers; outer primers, forward:CE21-1, GACTTACTCTTGATTGTAACGAGGA (8531-8555), reverse: CE22-1; ACTTGAAGCACTCAAGGCAAGCT(9602-9629); inner primers, forward, N1:GTAG CTGAGGGACAGATAGGGTTAT (8678-8703), reverse, N2:CACTCAAGGCAAGCTTTAT-TGAGGC (9591-9621). Primer locations were relative to HIV-1 NL4-3. After the first denaturation at 94°C for 1 min, 30 cycles were done under the condition of 94°C for 1 min, 50°C for 1 min, and 72°C for 90 sec, followed by a final extension at 72°C for 10 min. The second PCR was

done with 5 ul of the first PCR product. Cycling condition was the same as above, except 60°C for 1 min. PCR reaction (50 ul) was 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl2, 200 uM each dNTP, 20 pmol of each primer, 1.25 Units AmpliTaq polymerase (Perkin-Elmer Cetus), and denatured DNA (10 ul). The second PCR product (943 bp) were analyzed on 1.5% agarose gels and visualized by ethidium bromide staining. The second PCR products were purified and then used for direct sequencing. Sequencing primers were N1 and N2. Sequencing reactions was done using T7 sequenase v2.0 (Amersham Life Science, USA).

A third PCR was done for a part of the samples (87-05; April 1999, 90-01; January 2002, 92-16; December 1996, 92-mk; December 2001, 91-20; March 2002). The first PCR reaction mixture was buffer 2 ul, dNTP 0.6 ul, primer CE21 (forward) & CE22 (reverse) each 0.6 ul, Taq polymerase 0.1 ul, and D.W 11.1 ul. Thirty cycles were done under the conditions of 94°C for 1 min, 50°C for 1 min, and 72°C for 2 min, followed by a final extension at 72°C for 10 min. The second PCR reaction mixture was PCR buffer 5 ul, dNTP 1.5 ul, primer N1 (forward) & N2 (reverse) each 1.5 ul, Taq polymerase 0.1 ul, and D.W 30.2 ul. Thirty cycles were done under the condition of 94 for 1 min, 55°C for 1 min, and 72°C for 90 sec, followed by a final extension at 72°C for 10 min. The third PCR reaction mixture was PCR buffer 5 ul, dNTP 3 ul, primer N-E3 (forward) & N-E4 (reverse) each 5 ul, Taq polymerase 0.1 ul, and D.W 11.1 ul. Thirty cycles were done under the condition of 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min, followed by a final extension at 72°C for 10 min.

CD4+ T cell count

CD4+ T and CD8+ T cell counts were measured by FACScan (Becton-Dickinson, Calif., USA) flow cytometer after staining of PBMC with phycoerythrin and fluorescein isothiocyanate (FITC)-conjugated antibodies for CD4 and CD8 antigen (Becton-Dickinson) (3).

Viral load:

Quantitative analysis of plasma viral RNA loads was performed with the Amplicor HIV-1 Monitor assay (Roche Diagnostics Systems) according to the manufacturer's instructions.

Nucleotide sequence accession numbers.

Representative HIV-1 *nef* sequences obtained from each patient have been submitted to the GenBank sequence database and have been assigned accession numbers Z98019 (KU1)-Z98035

(KU17), AF224507, AF238268-AF238278, AF272003-AF272009, AF462693-AF462793 (except AF462711and AF462738), and AY121440-AY121480.

Statistical analysis

Data were expressed by mean standard deviation. The relationship between Nef progression scores, annual fall of CD4+ T cell count, plasma viral titers, absolute CD4+ T cell counts, duration of NRTI, and KRG-intake were tested by using Pearson's correlation coefficient. Chi-square tests were used for data analyses between variables.

Results

Defective nef genes are frequent

To investigate whether the *nef* gene could be affected by long-term intake of KRG, nested PCR amplication was performed using PBMCs obtained from 70 patients The follow-up period from diagnosis to last NefProg score was 8.82.9 years. To find whether there is a difference in the frequency of inactivating point mutations or small deletions among the 3 progression groups, we classified 70 patients (17 in SP, 17 in RP, and 36 in TP, respectively) (Table 2) (slide presentation).

We determined and analyzed 200 *nef* sequences to investigate whether specific amino acid substitutions in the *nef* gene are associated with progression. The deduced amino acid sequences are shown in Fig. 1 (slide presentation). We detected various deletions in the *nef* gene in 21 patients (30%), not including 5 premature stop codons (a patient showed together isoleucine as non- M-initiation codon). Nine patients showed defects of more than 2 kinds among premature stop codon, deletions (at 2 different sites) and non-M-initiation codon like isoleucine (I). All 10 patients with premature stop codons were treated with KRG for more than 3 months and 6 of them also showed isoleucine as an initiation codon or deletion (Table 1). However, 10 patients (89-15, 89-26, 90-05, 90-38, 90-39, 90-54, 92-05, 92-06, 92-38, and 92-48) showed a defective gene in only 1 out of many PCR products. The number of patients with repeated gross deletion was significantly higher in order of SP (90-18, 93-01, and 93-60: 18%), TPs (91-14; 3%), and RPs (0%) ($P<0.05$).

Regarding the deletion, gross deletion $\geq$ 5 amino acids was detected in 11 other patients (89-02, 89-26, 90-18, 90-39, 91-14, 91-23, 92-05, 92-06, 92-75, 93-01, and 93-60). However, the deleted sequences were only a third or a fourth of PCR products except in 4 patients (90-18, 91-

14, 93-01 and 93-60). Another 5 patients showed deletions in the last cysteine singly or combined with a deletion in another region. Specifically, patient 91-11 showed consistent deletion of 4 amino acids (codons 8-11 in Fig. 1) (slide presentation). Two patients (87-05 and 89-31) showed consistent deletion of 3 amino acids (codons 9-11) at the same region. Patient 89-18 showed 2 amino acids (codons 63-64). Two patients (90-14 and 00-179) with deletions of 2 amino acids, which is a characteristic of CRF01_AE, were not counted as deletions.

Correlation between progression to AIDS and duration of KRG-intake

First of all, there was a significant correlation between survival duration (8.8±3 years) and duration of KRG-intake (37±38 months) ($P<0.001$). We defined NefProg score based on Korean *nef* amino acid sequences (Table 3) because the positions showing statistical significance were different from a previous report (19). There were significant correlations between the last NefProg score and CD4+ T cell count ($r = -0.208$, $P<0.05$), and annual decrease in CD4+ T cell count ($r=0.346$, $P<0.01$) in 70 patients. In addition, there were significant correlations between KRG-intake (37±38) and annual decrease in CD4+ T cell count ($r= -0.323$, $P <0.01$), and CD4+ T cell count itself ($r=0.229$, $p<0.05$). Furthermore, there was also a small significance between the NefProg score and the duration of KRG-intake in only SP and RP ($r=-0.281$, $P=0.067$). However, there was no significant correlation between NRTI duration and annual decrease in CD4+ T cell

Table 3. Differences between Nef sequences derived from different progression groups

	No. (%) of occurrences in:				P value	
	SPs (n = 17)	TPs (n = 36)	RPs (n = 17)	Subtotal (n = 53)	SPs vs RPs	SPs vs Subtotal
PxxP	0 (0)	3 (8)	2 (12)	5 (9)	0.061	0.171
15 T	13 (81)	17 (47)	2 (12)	19 (36)	<0.001	<0.01
a	3 (18)	10 (28)	9 (53)	19 (36)	<0.05	0.16
39 Q	16 (94)	21 (58)	9 (53)	30 (57)	<0.05	<0.01
k	0 (0)	4 (11)	4 (24)	8 (15)	<0.05	0.089
54 T	4 (24)	1 (3)	0 (0)	1 (2)	<0.05	<0.01
d	2 (12)	17 (47)	5 (29)	22 (41)	0.171	<0.05
157 T	16 (94)	25 (69)	10 (59)	35 (66)	<0.05	<0.05
n	1 (6)	12 (33)	4 (35)	18 (34)	<0.05	<0.05
169 N	16 (94)	20 (56)	10 (59)	30 (57)	<0.05	<0.01
s	0 (0)	10 (28)	6 (35)	16 (30)	<0.05	<0.05
182 v	7 (44)	23 (64)	12 (71)	35 (66)	0.084	0.069
E	5 (29)	3 (8)	1 (6)	4 (7)	0.072	<0.05

Although one additional N-terminal PxxP motif did not show statistical significance, it was counted as feature of progression marker because it was almost always detected in late stage patients.

(4157/ul) (r=-0.084, P=0.244), and the count itself (r=0.053, P>0.05). RNA copy was in part determined. There was also a significant correlation between the NefProg score and the plasma RNA copy in 20 HAART-naive patients except nonsubtype B 3 patients (n=33, r = 0.353, P<0.05).

Features of Nef sequences associated with progression.

In regard to NefProg score, our data showed consistency with Kirchhoff's report (19). However, there was great difference in some amino acid compositions because the Korean variant of subtype B in the *env* and *nef* gene is quite distinct from worldwide consensus (21). Thus, the NefProg score is defined so that the number of features more typical for nonprogression (T15, Q39, T54, N169 and E182) was subtracted from the number of features more frequently observed in progression (A15, K39, D54, N157, S169, and V182 and one additional PxxP) (Table 3). In contrast to Kirchhoff's report, there was no significance in positions 51, 102, 163, and 170 that showed significant difference according to progression groups. One additional N-terminal PxxP motif was detected in 5 patients. Those 2 patients were terminal AIDS patients with CD4+ T cell counts of less than 200/ul. Thus, although its distribution did not show statistical difference among groups (Table 3), we counted it as a feature of a progressive marker.

Change of NefProg score in 47 patients

Forty-seven patients were sequenced twice during 57 30 months. The NefProg score increased and decreased in 11 patients. During the period, the duration of KRG-intake was 5044 months in 47 patients, and CD4+ T cell count decreased from 350±1246/ul to 295±236/ul (P>0.05) and NefProg score increased from -0.79±3.5 to -0.74±3.4 (P>0.05). And the extent of change of the NefProg score was 0.91±1.06.

On the other hand, there were significant differences between hemophiliacs with KRG-intake for 46±47 months and nonhemophiliacs with KRG for 51±43 months. The NefProg score increased in 5 (45%) of 11 hemophiliac patients, whereas the increase was in 6 (17%) of 36 nonhemophiliacs (P<0.05). CD4+ T cell count increased from 313±164/ul to 329±199 in hemophiliac patients, whereas it decreased from 361±266 to 285±247/ul in nonhemophiliacs (P<0.05). Actually, 5 of them belonged to SP in this study. The NefProg score significantly increased from -2.27±2.8 to -1.36±2.9 (P<0.05) for 60±22 months in hemophiliacs, whereas it decreased from -0.333.56 to -0.553.58 in nonhemophiliacs for 5632 months (P>0.05). The extent of the change of the NefProg score was more (1.18±1.31) in hemophiliacs than that (0.86±1.0) in nonhemophili-

acs (P>0.05). The greatest change (from -4 to -1) in the NefProg score was also detected in hemophiliac patient 92-06 over 50 months. The same phenomenon in NefProg score was also found in Kirchhoff's report (19).

Four cases with repeated gross deletions in nef/LTR

A 26-year-old female patient (93-60) was diagnosed with HIV-1 infection in 1993. The patient was infected with HIV-1 by her husband (89-17) in March 1993. She had a primary infection syndrome, such as fever, after first sexual contact with her spouse in March 1993. Her husband, who engaged in homosexual activity, had been diagnosed with HIV-1 in early 1989. She gave birth to an uninfected newborn in March 1998. In April 1994. the first CD4+ T cell count and CD4+ T cell to CD8+ T cell ratio were 475/ul (27.7%) and 0.84, respectively . In November 1995, the second CD4+ T cell count and CD4/CD8+ T cell ratio were 394/ul (25.7%) and 1.25, respectively. The CD4+ T cell to CD8+ T cell ratio were maintained above 1.1 up to June 1999. In September 2002, the last CD4+ T cell count and CD4/CD8+ T cell ratio were 420/ul (31.1%) and 0.97. respectively. However, her husband without deletion in the *nef* gene has showed a gradual decrease in CD4+ T cell from 384/ul (17%) in January 1990 to 106/ul (10.9%) in January 2002. Although plasma RNA copy in 93-60 should be "of". was high (14,655/ml) in November 2000, and there has been no tendency of decrease in CD4+ T cell count for 114 months. Patient 93-60 showed various gross deletions along with wild type. *Nef* sequence was wild type in 2 clones by a PCR product using PBMC obtained in November 1996. We do not know exactly whether there was no gross deletion at that time due to limitation of samples and because only 1 PCR product was obtained. Samples were not available before June 1999. We began to obtain grossly deleted *nef* sequences (Δ410 bp) in June 1999 (GenBank accession no. AF462781). The portion of deletion was the nearly central part of the *nef* gene. Another PCR product (AF462782) showed a deletion of the last cysteine. For the sample obtained in November 2000, 4 separated PCR products were sequenced. There was a gross deletion in 2 of 4 (AF462783, Δ94 bp from 9024 to 9,117 relative to HIV-1 NL4-3; AF462784, Δ355 bp;288 bp in the *nef* region and 66 bp in LTR after the end of the *nef* coding gene). There were also 2 premature stop codons before the deleted region (codons 13 and 71 in Fig. 1) (slide prsentation). Although the third product was a strong short band compared to the wild *nef* gene and showed terminal *env* sequences, there were no *nef* sequences. The fourth showed the wild-type *nef* gene (AF462785). One of 4 normal size PCR products obtained from the sample in July 2001 was sequenced (AF462786). One wild-type

nef (AY121475) and 3 short PCR products from the sample obtained in November 2001. We could not obtain a short PCR band in 4 PCR products from the sample obtained in January 2002. The NefProg score has been maintained -4 over 5 years. In contrast, although her husband's sequences were very similar to hers, there was no deletion in 3 PCR products. The NefProg score increased from -3.5 (mean of two sequences) to -4 for 63 months. Although we could not find any causal relationship or specific change between a defect in *nef* and KRG-intake, long-term intake of KRG showed slow decrease in CD4+ T cell count for 10 years.

Patient 90-18 (SP) was diagnosed with HIV-1 infection in May 1990. He is a LTNP who has maintained a CD4+ T cell count of more than 500/ul till April 2002. He has taken KRG since 1991. The total amount of KRG supplied is 11,382g. *Nef* sequences in June 1993 were wild type in all 3 PCR products. In July 1997, 2 of 3 PCR products showed gross deletion at 2 different sites (codons 64-65 and 94-132: total deletion of 122 bp). There was a deletion of two amino acids in 2 of 2 separated PCR products in April 2002.

Although patient 93-01 had presumed infection in 1988, he was diagnosed with subtype D HIV-1 infection in December 1992. Although his CD4+ T cell count has shown some fluctuation, it has gradually increased from baseline 199/ml to 669/ul in August 2002 (Fig. 2)(slide presentation). Despite no antiretroviral therapy, HIV-1 plasma RNA copy was 940, 2,147, and 1,571 copies/ml in August 1997, April 1998, and March 2001, respectively, which were below therapy guidelines. Because the patient was infected with subtype D, although the NefProg score was high, it decreased from 4 to 3. Interestingly, the 15 bp deletion in the N-terminal region (codons:8-12, Δ15 bp) in all 7 PCR products has been detected from July 6th, 1993, (AF462775) to March 2002 (GenBank accession nos. AF462776, and AF462777). In the sample of July, 1993, 1 premature stop codon was detected (AF462775). There was no deletion in *env* C2-V3 (GenBank accession no. Z92594) and reverse transcriptase gene (AF273205) (31). Although this patient has taken KRG with a few interruptions since April 1993, we are not sure that the deletion in the *nef* gene might be caused by long-term intake of KRG. (The amount of KRG supplied until now was 8,100g. He has also taken KRG that he purchased for himself.) However, the increase in CD4+ T cell and low viral load might have some correlations with both of long-term intake of KRG and subtype D HIV-1 (1).

Patient 91-14 showed a deletion of 2 amino acids (codons: 8 and 9) in the myristylation portion 3 times (Dec, 1993, Dec 1996, and Mar 2000). Interestingly, the deletion began to expand from 3 to 9 amino acids (codons: 4-12) on July 2002. Although the NefProg score did not change for

103 months, the patient has strictly taken KRG since November 1991. This case showed us the possibility that deletion in the *nef* gene could have occurred because of the long-term intake of KRG. The total amount of KRG supplied was 8,790g and he personally purchased KRG.

A clear evidence for the effect of KRG intake.

The blood donor patient 91-20 was a 29-year-old man who was seropositive for HIV-1 in June 1991, and his CD4+ T cell count was above 700/ul (Fig. 3). He had a history of blood donation in October 28th, 1989. A retrospective study showed that his two recipients (Patients 91-22 and 91-23) were infected with HIV-1 in December, 1989. He also had an admission history of fever, chill, and sweating in 1988. However, he was not tested for HIV at that time. Plasma RNA copy number was 5,825/ml in July 1997 and a viral culture was done for PBMC samples sampled in July, 1997. Culture supernatant was positive for p24 antigen from culture day 9. The biological nature of the virus was the nonsyncytium- inducing type (6). Plasma RNA copy number had maintained at 5,649/ml in March 2001, below the therapeutic guidelines. CD4+ T cell count has been maintained above 500/ul as of August 2002 In regard to therapy, he has taken KRG, 5.4 grams daily, since December 1991, although there were several interruptions. He is definitely a long-term nonprogressor (GenBank accession no. AF224507). The total amount of KRG supplied was 8,856g and he personally purchased KRG.

In contrast, the recipient of his blood was patient 91-22, a 7-year-old boy who in December 1989 was transfused due to a traffic accident. Although 91-22 has been treated with antiretroviral drugs (3TC and IDV) since December 1999, his last CD4+ T cell count and plasma viral load in December 2001 was around 213/ul and 1,470 copies/ml, respectively. The second recipient, 91-23, was a 16-year-old male in December 1989. Patient 91-23 has been treated with zidovudine (daily 800 mg) since April, 1993. He showed an increased CD4+ T cell count compared to baseline value until March 1993. Thereafter, we continuously found resistance mutations (67N, 69N, 70R, and 219Q) to ZDV (8). In November 1999, ZDV was replaced with 3TC. As of December 2001, CD4+ T cell count and viral load were 152/ul and 366,197 copies/ul (Oct. 2000), respectively. In regard to NefProg score, there was no increase in patient 91-20 over 8 years (-3 on March 2002) and in 91-22 over 2 years (-3 on December 2001). However, in 91-23, it increased 3 points from -3 to -1 over 9.5 years (-1 in July 2002) despite antiretroviral therapy. This is a good evidence on which long-term intake of KRG is definitely beneficial for HIV-1 infected individuals although precise action mechanism must be elucidated in the future study.

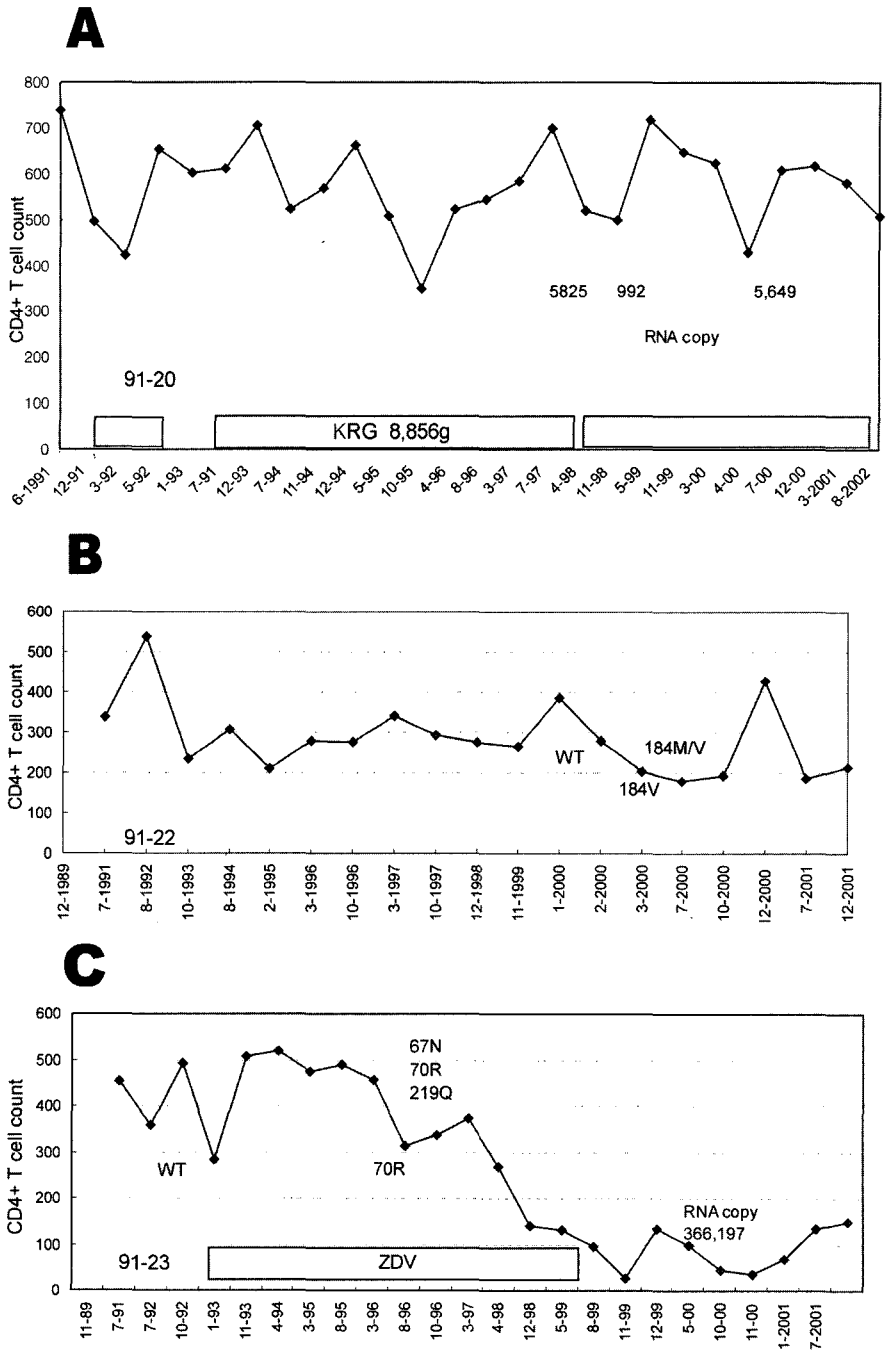

Fig. 3. Month from HIV infection.

Table 4. Nef amino acid variations observed during KRG intake

No.	Date	CD4+	Months		Amino acid at position									Nef	RNA
patient	(m-yr)	T cells	KRG	PxxP	15	39	51	102	157	163	169	170	182	Prog	copy
87-05	9412	178	6	-	a	K	N	H	N	S	S	L	m/E	-3	
	9704	98		-	a	K	N	H	N	S	S	L	k/m	-2	22,452
	9904	120	58	-	a	K	N	H	N	S	S	L	m	-1	
	0003	136	85	-	a	K	N	H	N	S	S	L	m	-1	10,838
88-17	9310	1244		-	K	r	t	Y	N	S	C	q	V	3	
	9804	550		-	K	r	t	Y	N	S	C	q	V	3	50
	0004	365		-	K	r	t	Y	N	S	C	q	I	3	
	0010	322	110	-	K	r	t	Y	N	S	C	q	I	3	34,594
89-17	9610	281		-	N/T	Q	N	W	t	c	n	L	E	-1	
	0003	214	16	-	T	Q	N	W	t	c	n	L	E	-1	
	0201	106	38	-	T	Q	N	W	t	c	n	L	E	-1	162,000
90-01	9902	299		-	a	Q	N	Y	t	c	n	L	V	2	
	0202	216	35	-	T	Q	N	W	t	c	n	L	V	0	97800
90-05	9310	272		-	T	Q	N	Y	t	c	n	L	V	0	
	0002	216		-	T	Q	N	Y	t	c	n	L	V	0	915
	0102	169	124	-	T	Q	N	Y	t	c	n	L	V	0	4,580
90-18	9306	893		-	T	Q	N	H	t	S	n	L	V	-2	
	9707	323		-	T	Q	N	H	t	S	n	L	m	-1	11,241
	0003	788	76	-	T	Q	N	H	t	S	n	L	m	-1	10,666
90-50	9309	838		-	T	Q	N	Y	t	S	n	L	V	-1	
	0107	523	77	-	T	Q	N	Y	t	S	n	L	V	-1	47,500
90-52	9108	198		-	T	Q	N	Y	t	c	n	L	E	-1	
	9612	189		-	T	Q/r	N	Y	t	c	n/S	L	E	-1	
	0102	260	104	-	T	Q	N	Y	t	c	n	L	E	-1	256,919
91-15	9305	446		-	T	Q	N	Y	t	c	n	L	V	0	
	9701	252	36	-	T	Q	N	Y	t	c	n	L	V	0	
91-20	9412			-	T	Q	N	Y	t	c	n	L	V	0	
	9707	701		-	T	Q	N	Y	t	c	n	L	V	0	
	0203	597	123	-	T	Q	N	Y	t	c	n	L	V	0	
91-29	9412	600	22	-	T	Q	N	H	t	c	n	q	V	1	
	9611	921		-	T	Q	N	H	t	c	n	q/L	V	0	
	9703	860	122	-	T	Q	N	H	t	c	n	L	V	-1	10,330
92-13	9401	202		-	T	Q	N	H	t	c	n	L	m	0	
	9910	282		-	T	Q	N	H	t	c	n	L	m	0	
	0102	321	60	-	T	Q	N	H	t	c	n	q	m	2	3765
92-16	9612	309		-	T	Q	N	H	t	S	n	L	E	-3	4606
	0012	260	108	-	T	Q	N	Y	t	c	n	L	V	0	4146
93-01	9307	325	3	-	a	K	t	W	N	c	C	q	K	4	940
	0011	708		-	a	r	t	W	N	c	C	q	K	5	
	0107	892		-	a	r	t	W	N	c	S	q	K	5	
	0203	1000	95	-	a	r	t	W	N	c	C	q	K	5	1571

Table 5. Nef amino acid variations observed during progression and HAART

No. patient	Date (m-yr)	CD4+ T cells	No.of clones	PxxP	Amino acid at position 15	39	51	102	157	163	169	170	182	Nef Prog
87-03	9412	178			T	K	N	H	N	S	S	q	m	-1
	0003	203(6)			T	r	N	H	t	c	C	q	m	2
88-11	9612	98			K	K	N	Y	N	S	S	L	V	-2
	0001	271			D	K	N	w	N	S	S	L	V	-2
89-02	9409	270			Q	r	t	Y	N	c	S	q	m	5
	0107	71			Q	r	t	Y	N	c	S	q	m	5
89-18	9603	88			T	Q	N	H	t	S	n	L	V	-2
	0012	114			T	Q	N	H	t	S	n	L	V	-2
89-26	9310	240			a									
	9609	310			a	Q	N	Y	A	c	H	q	V	2
	0003	109(28)			a	Q	N	Y	t	c	n	q	V	4
90-38	9905	357			a	K	t	H	N	c	S	q	V	3
	9911	401			a	K	t	H	N	c	S	q	V	3
	0203	548(187)			a	K	t	H	N	c	S	q	V	3
90-39	9310	436			T	Q	N	Y	N	S	n	L	V	-2
	0002	63			T	Q	N	Y	t	S	n	L	V	-1
	0203	240			T	Q	N	Y	t	S	n	L	V	-1
91-11	9911	121			a	T	t	Y	t	S	C	q	R	4
	0103	15			a	T	t	Y	t	c	C	q	R	5
	0201	46			a	T	t	Y	t	c	C	q	R	5
91-14	9312	138			N	K	N	Y	N	c	S	q	E	0
	9612	266			N	K	N	Y	N	c	S	q	E	0
	0003	306			N	K	N	Y	N	c	S	q	E	0
91-20	9412				T	Q	N	Y	t	c	n	L	V	0
	9707	701			T	Q	N	Y	t	c	n	L	V	0
	0203	597			T	Q	N	Y	t	c	n	L	V	0
91-22	0001	387			T	Q	N	Y	t	c	n	L	V	0
	0112	213			T	Q	N	Y	t	c	n	L	V	0
91-23	9210	492			T	Q	N	Y	t	c	n	L	V	0
	0010	46		+	S	Q	N	Y	t	c	n	L	V	2
92-05	9706	58			a	Q	N	H	t	c	n	L	V	1
	0001	507			a	Q	N	H	t	S	n	L	E	-1
	0112	590			a	Q	N	Y	t	c	n	L	V	2
92-31	0103	653(97)			T	Q	N	H	t	S	n	L	m	-1
92-38	9604	243			T	Q	N	Y	t	c	n	L	V	0
	0102	334			T	Q	N	Y	t	c	n	L	V	0
92-41	9612	97			a	Q	N	Y	t	c	n	L	V	2
	0011	38			T	Q	N	Y	t	c	n	M	V	2
93-12	9306	296(191)			T	Q	N	Y	t	c	n	L	V	0
	0011	343			a	Q	N	Y	t	c	n	L	V	2
98-86	0110	594		+	T	K	t	H	N	c	S	q	V	2
	0202	175(15)			?	?	?	H	N	c	S	q	V	2
01-LHS	0102	209			a	Q	N	Y	t	c	n	q	m	5
	0112	715			a	Q	N	Y	t	c	n	q	m	5

Long-term intake of KRG causes a change from A or D to T at position 54 and decreases Nef-Prog score

Interestingly, we found that the natural progressive change at position 54 was from alanine (A) to aspartic acid (D) in 2 couples (93-60 and 89-17, 91-05 and 90-54) and 2 patients (90-14 and 90-18) (Table 5) (slide presentation). In addition, patient 91-23, whose amino acid was alanine at early infection, was infected with HIV via transfusion from patient 91-20 continuously showing threonine. Patient 91-20 has taken KRG for 114 months (Table 4). In contrast, the amino acid at position 54 changed from A or aspartic acid (D) to threonine (T) in 3 patients (90-05, 90-18. and 91-15) treated with KRG for a prolonged period and it was already threonine in another 3 patients treated with KRG. Patient 90-05 in particular showed the best compliance for KRG since late 1991. He showed mixed amino acids (D; GenBank accession no. AF462709 and A; AF462710) at position 54 in October 1993, and one (GenBank AF462710) of them also showed other defects (initial isoleucine, 3 premature stop codons, and deletion of 1 of 2 NF-B in LTR), alanine in May 1995. Thereafter the position changed into threonine (characteristic of slow progression in this study) up to August 2002 although there were re-appearance of T15A and A37D in only March 2002. Patient 90-18 showed the same phenomenon for 9 years and also showed gross deletion of *nef* in July 1997.

On the other hand, the *nef* gene in 15 patients was sequenced before and during HAART. The NefProg score increased in a hemophiliac patient, decreased in 3 patients, and showed no change in 11 patients (Table 6) (slide presentation) (from 0.93 ± 2.8 to 0.71 ± 2.7, $P = 0.213$). Although CD4+ T cell count increased from <200/ul to >500/ul in 2 patients (90-38 and 92-05) because of HAART, there was no decrease in the NefProg score in accordance with the increase in CD4+ T cell count. Another 2 patients (not included in this study) who had terminal AIDS with opportunistic infections recovered CD4+ T cell count to more than 500/ul. Despite that significant increase in CD4+ T cell count, NefProg score did not decrease. There was a decrease in NefProg score in 2 patients (87-03 and 92-41) treated with KRG for more than 30 months, whereas there was no decrease in NefProg score in the 6 patients treated with HAART only.

Discussion

Panax ginseng C. A Meyer is an herbal root, which has been known in China for more than 2000 years. Many scientific investigations have been performed on the active ingredients and

their functions since the late 1960s. In regard to antimicrobial effect, Song *et al.* reported that ginseng treatment reduces bacterial load and lung pathology in chronic *Pseudomonas aeruginosa* pneumonia in rats. Ginseng is now one of 12 medicinal herbs commonly used in America. A double-blind study in normal human volunteers revealed a significant increase in neutrophil function, CD4+ T cell counts and NK-function in individuals taking ginseng compared to those taking placebo. It is particularly interesting that the immunostimulating effect of acidic polysaccharide (Ginsan) from ginseng root is blocked in the presence of anti-interleukin (IL)-2 and anti-IFN-*r*. In combination with HAART, IL-2 has been tried for HIV-1 infected individuals (7).

First, deletion in the *nef* gene was detected in 21 (30%) of 70 patients. To my knowledge, this frequency is the highest among the general HIV-infected population. In this study, a total of 20 (10%) among 200 *nef* genes from 70 patients were grossly (5 amino acids) defective. This frequency is more than 3 times that (2 of 88; 2.3%) of another report that had a similar population structure (25). To our knowledge, there is no report on the clinical significance of the number of deleted amino acids and definition of gross deletion. We detected a gene defect in 17 SP in 6 patients (37.5%). In 3 patients (18.7%) it was grossly deleted. This frequency data contrasts sharply with the data of Huang et al (0/10 LTNPs (17) and Rhodes et al (4.3%; 3 in 70 LTNPs) (27).

With regard to KRG-intake on gross deletions 5 amino acids in the *nef* gene, 3 of 4 patients with gross deletions have maintained or increased CD4+ T cell count for 9 years, although patient 91-14 has been treated with KRG and HAART. Apart from gross deletions in patients 93-01 and 93-60, the appearance in patients 90-18 and 91-14 of gross deletions in 1997 after 3 wild-type sequences from 1993 and an extended deletion of 2 to 3 times to 9 amino acids raises the possibility that the deletion might result from long-term KRG-intake. It is known that A29V (A27 in Fig 1 of this paper) means loss of CD4 downregulation (26). Patient 90-01 in SP showed deletion at that position.

Although we could find only a few patients with threonine (T) at position 54 in other reports (12), there is no report on the change (Alanine;A-> aspatic acid;D-> T: good prognosis). The change seems to be the same with that at position 29 (T->A: bad prognosis) although the outcome is opposite. Two of 46 patients in our previous report (18) also showed T at position 54 although it is unknown whether they were treated with KRG or not. Although we are not sure that these changes result from long-term intake of KRG because there were not enough cases, we need to clarify whether there is a causal relationship between long-term intake and the changes

from A or D to T at position 54 in the future.

We have encountered 2 couples: the deletion in *nef* in 93-60 originated from her spouse with wild-type *nef*, and the deletion of 3 amino acids in patient 01-sj originated from her spouse with the same deletion in the same region. We need to have answers to understand the interaction between the host and HIV.

During 57±30 months, the range of the NefProg score change was 0.91±1.06 in 47 patients. This small change in the NefProg score might be caused by the small decrease in CD4+ T cell count (29/ul for 77±23 months in Table 4, $P>0.05$) due to the long-term intake of KRG (85±33).

Phenomenon like the NefProg score were already detected in a previous report on the *env* gene in 65 patients (5). Fifty-four of the 65 patients were included in this study. The increase in the NefProg score reflects progression as if the increase in the positive charge in the V3 loop means an increase of virulence due to the change from NSI to SI (33). Based on our studies, a local government in Korea has spplied KRG to about 40 HIV-1 individuals since September 2001, and the increase in CD4+ T cell count bhas been observed.

If HAART could give hope of a cure for an HIV infection, the NefProg score should have been reverted by HAART. In other words, HAART has to change the nature of the virus, such as bring about a decrease in the NefProg score.

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Seventy patients consisted of seventeen SPs, 17 RPs, and 36 TPs. Twenty-one of the 70 patients (200 PCR products) showed various deletion deletions in *nef* sequences. [a]Number of sequences, [b]deletion of one amino aicd, [c]deletion of last cysteine, [d]polymorphism in variable length region, [e]untypable, [f]an additional N-terminal PxxP, [g]initial isoleucine (I) instead of methionine, [h]premature stop codon, RTI+H: RT inhibitors and then highly active antiretroviral therapy. Overseas; overseas sailor. Prostitute; female sex worker who worked at sex shop next to military base of USA army in Korea. Hetero: heterosexual contact, Annual fall was calculated by which the difference between the first CD4+ T cell counting and the last counting before two drug combination therapy or HAART was divided by the follow up months and then times 12., ΔG: gross deletion, CRF: circulating recombinant form, *the count just before HAART

이름	Date	CD4										Nef Prog	RTI	KRG	HAART	RNA copy	subtype
			PxxP	15	39	51	102	157	163	169	170	182						
87-03	9412	178		T	K	N	H	N	S	S	q	m	-1	0	17			
	0003	203(6)		T	r	N	H	t	c	C	q	m	2	40	32	HAART		
87-05	9412	178		a	K	N	H	N	S	S	L	m/E	-3	0	6			
	9704	98		a	K	N	H	N	S	S	L	k/m	-2				22,452	
	9904	120		a	K	N	H	N	S	S	L	m	-1	0	58			
	0003	136		a	K	N	H	N	S	S	L	m	-1	0	85		10,838	
	011205																	
87-08	9505	41		T	K	t	H	N	c	S	q	I	1	41	14			
87-09	0109	1308		R	r	N	Y	N	S	C	q	V	0	39	0		<147	
88-01	020427							N	S	S	q	V					정경훈	
88-04	9911	110		a	A	t	H	t	S	C	q	m	4	16	6			Untyped
88-11	9612	98		K	K	N	Y	N	S	S	L	V	-2	0	15			
	0001	271		D	K	N	w	N	S	S	L	V	-2	9	15	HAART	PCR ok	
88-12	9310	49		a	K	N	Y	N	S	S	L	V	-1	24	9			
	9707	1		a	K	N	Y	N	S	S	L	V	-1	24	10			
88-17	9310	1244		K	r	t	Y	N	S	C	q	V	3					A
	9804	550		K	r	t	Y	N	S	C	q	V	3				50	
	0004	365		K	r	t	Y	N	S	C	q	I	3					
	0010	322		K	r	t	Y	N	S	C	q	I	3	0	110		34,594	I clear
88-22	9512	392		Q	r	N	Y	N	c	S	q	V	2	0	21			AG
89-02	9409	270		Q	r	t	Y	N	c	S	q	m	5	38				
	0107	71		Q	r	t	Y	N	c	S	q	m	5	46	19	HAART		untyped

89-05	9608	417		a	Q	N	Y	t	S	n	L	E	0	0	0			
89-07	9412	6		a	Q	N	Y	t	S	n	L	V	1	9	7			
89-10	0009	297		Q	K	N	Y	t	c	n	L	V	1	0	12		345,654	AF462702
89-12	0012	53		a	Q	N	Y	t	c	n	L	V	2	0	0			
89-15	9701	14	PxxP	T	Q	N	Y	t	c	n	L	V	1	36	40			Untyped
																	4641	
89-17	9610	281		N/T	Q	N	W	t	c	n	L	E	-1					
	0003	214		T	Q	N	W	t	c	n	L	E	-1	0	16			
	0201	106		T	Q	N	W	t	c	n	L	E	-1	0	38		162,000	
89-18	9603	88		T	Q	N	H	t	S	n	L	V	-2	0	16			
	0012	114		T	Q	N	H	t	S	n	L	V	-2	20	19	HAART		
	011228																	
89-26	9310	240		a											0	0		
	9609	310		a	Q	N	Y	A	c	H	q	V	2	0	0	0		
	0003	109(28)		a	Q	N	Y	t	c	n	q	V	4	12	0	HAART	439	
89-31	9306	366		a	K	t	Y	N	c	S	q	V	4	0	0			
90-01	9902	299		a	Q	N	Y	t	c	n	L	V	2					
	0202	216		T	Q	N	W	t	c	n	L	V	0	0	35		97800	
90-05	9310	272		T	Q	N	Y	t	c	n	L	V	0					
	0002	216		T	Q	N	Y	t	c	n	L	V	0				915	
	0102	169		T	Q	N	Y	t	c	n	L	V	0	0	124		4,580	
90-06	9510	210		T	r	N	W	N	V	S	q	Q	0	29	47			
90-12	9703	226		T/a	Q	N	Y	t	R	n	L	E	-2/0	6	11			
	9903	8		a	Q	N	Y	t	c	n	L	E	1	12	14			
90-51	0108	447		Q	K	N	Y	N	c	S	q	R	1				591	E
	0112	366		Q	K	N	Y	N	c	S	q	R	1	0	10		3,710	
90-18	9306	893		T	Q	N	H	t	S	n	L	V	-2					
	9707	323		T	Q	N	H	t	S	n	L	m	-1				11,241	
	0003	788		T	Q	N	H	t	S	n	L	m	-1	0	76		10,666	
90-22	9307	274		a	Q	N	Y	t	c	n	L	E	1	24	18			
	9605	283		N	Q	N	Y	t	c	n	L	V	1	56	60		오염 가능성	
90-35	0005	24	PxxP	a	r	N	Y	N	V	S	L	V	1	0	97			
	0102	61	PxxP	a	r	N	Y	N	V	S	L	V	1	0	101			
90-36	9306	547	PxxP	K	r	t	Y	N	S	S	q	V	4	0	4			
90-37	9911	209		a	T	t	Y	t	c	C	q	R	5	0	8			

90-38	9905	357		a	K	t	H	N	c	S	q	V	3			Seq111	
	9911	401		a	K	t	H	N	c	S	q	V	3	0	15	256,250	
	0203	548(187)		a	K	t	H	N	c	S	q	V	3	17	35	HAART	
90-39	9310	436		T	Q	N	Y	N	S	n	L	V	-2				
	0002	63		T	Q	N	Y	t	S	n	L	V	-1	0	90	240,000	
	0203	240		T		del		t	S	n	L	V				Haart	short
	0203	seq139		T	Q	N	Y	t	S	n	L	V	-1				
90-50	9309	838		T	Q	N	Y	t	S	n	L	V	-1				
	0107	523		T	Q	N	Y	t	S	n	L	V	-1	0	77	47,500	
90-52	9108	198		T	Q	N	Y	t	c	n	L	E	-1				
	9612	189		T	Q/r	N	Y	t	c	n/S	L	E	-1				
	0102	260		T	Q	N	Y	t	c	n	L	E	-1	0	104	256,919	
90-57	9306	226		T	r	t	Y	N	c	S	L	V	1	26	8		
	9512	81		a/T	r2	t2	Y2	N2	c2	S2	L2	V2	2/3	56	38		
	9912	13	PxxP	a									4	104	80		
91-05	9506	344		T	Q	N	Y	t	S	n	L	E	-2	51	43		
	0102	191		T	Q	N	Y	t	c	n	L	V	0	100	102	45,660	
	0111	204														122,000	
91-10	9911	121		a	T	t	Y	t	S	C	q	R	4	3	42		
	0103	15		a	T	t	Y	t	c	C	q	R	5	5	50		
	0201	46		a	T	t	Y	t	c	C	q	R	5		55	HAART	
91-14	9312	138		N	K	N	Y	N	c	S	q	E	0	21	25		
	9612	266		N	K	N	Y	N	c	S	q	E	0			AZT	
	0003	306		N	K	N	Y	N	c	S	q	E	0	96	122	HAART	400
	020125																
91-15	9305	446		T	Q	N	Y	t	c	n	L	V	0				
	9701	252		T	Q	N	Y	t	c	n	L	V	0	0	36		
91-16	9305	529		T	Q	N	Y	t	c	n	L	V	0				
	9605	296		T	Q	N	Y	t	c	n	L	V	0	0	2		
	011021																14800
91-20	9412			T	Q	N	Y	t	c	n	L	V	o			ann 109	
	9707	701		T	Q	N	Y	t	c	n	L	V	0				5825

	0203	597		T	Q	N	Y	t	c	n	L	V	0	0	123		5649	
91-21	9306	153		K/S	K	t	Y	N	c	S	Q	m/V	3/4	6	15			
91-22	0001	387		T	Q	N	Y	t	c	n	L	V	0					
	0112	213		T	Q	N	Y	t	c	n	L	V	0	19	0	HAART	1470	
91-24	9210	492		T	Q	N	Y	t	c	n	L	V	0	0	0			
	0010	46	PxxP	S	Q	N	Y	t	c	n	L	V	2	90	11	HAART	366,197	S clear
91-29	9412	600		T	Q	N	H	t	c	n	q	V	1	36	22			
	9611	921		T	Q	N	H	t	c	n	q/L	V	0					
	9703	860		T	Q	N	H	t	c	n	L	V	-1	68	122		10,330	
	020206	973																
91-32	9404	364		E	K	N	Y	t	S	C	q	R	1	0	14			
91-37	9605	520		T(2)	Q	N	Y	t	c	n	L	V	0					
	9910	200		a(2)	Q	N	Y	t	c	n	q	m	5	0	3			
92-01	9705	4		a	E	t	Y	t	c	n	L	V	4	0	0			
92-05	9706	58		a	Q	N	H	t	c	n	L	V	1	0	9			
	0001	507		a	Q	N	H	t	S	n	L	E	-1			HAART		E clear
				a	Q	N	del	del	del	del	del	del						
	0112	590		a	Q	N	Y	t	c	n	L	V	2	59	9	HAART	<50	
92-06	9306	>458		T	Q	N	Y	t	c	n	L	E	-1	0	0			
	941104																	
	9510	53	PxxP	a	Q	N	Y	t	c	n	L	Q	3	0				
	9708	2		a	Q	N	Y	DEL	DEL	DEL	DEL	DEL		4	4			
	9708	seq135		a	Q	N	Y	t	c	n	T	Q	3					
92-08	0011	142		T	Q	N	Y	t	S	n	L	V	-1	0	20		Subtype E	
92-11	9602	28	PxxP	T	Q	N	Y	t	c	n	L	V	1	43	38			
92-13	9401	202		T	Q	N	H	t	c	n	L	m	0					
	9910	282		T	Q	N	H	t	c	n	L	m	0					
	0102	321		T	Q	N	H	t	c	n	q	m	2	0	60		3765	
92-16	941006																	
	9612	309		T	Q	N	H	t	S	n	L	E	-3				4606	
	0012	260		T	Q	N	Y	t	c	n	L	V	0	0	108		4146	
92-19	9903	552		T	Q	t	Y	t	S	n	L	E	0				1440	
	0112	722		T	Q	t	Y	t	c	n	L	V	2	0	4		147	
92-10	9606	1082		T	Q	N	Y	t	c	n	q	m	3	0	5			
92-28	9306	368		a	Q	N	Y	S	c	n	L	V	1	0	8			
92-31	0103	653(97)		T	Q	N	H	t	S	n	L	m	-1	57	70	HAART	506	

92-32	9609	4		V	Q	N	Y	t	c	n	q	V	3	30	18		V clear	
92-38	9604	243		T	Q	N	Y	t	c	n	L	V	0	0	24			
	0102	334		T	Q	N	Y	t	c	n	L	V	0	41	26	HAART	368	
									·									
92-41	9612	97		a	Q	N	Y	t	c	n	L	V	2	13	32			
	0011	38		T	Q	N	Y	t	c	n	M	V	2	37	34	HAART	M clear	
92-42	9611	28		a	Q	N	Y	t	c	n	q	V	4	4	3			
92-48	9701	184		T	Q	N	Y	t	c	n	3L/q	V	0	32	43		A	
92-52	9603	189	PxxP	a	r	t	W	t	S	C	q	V	6	0	3			
92-62	0104	144		T	Q	N	W	t	c	n	q	V	2	21	16			
92-75	9704	280		T	Q	N	Y	t	c	n	L	m	1					
	0011	189		T/a	Q	N	Y	t	c	n	L	m	2	0	4			
	0103	229		a	Q	N	Y	t	c	n	L	m	3	0	7			
92-76	9611	314		T	Q	N	Y	t	c	T/n	L	V	-1	0	43			
93-01	9307	325		a	K	t	W	N	c	C	q	K	4	0	3		940	
	0011	708		a	r	t	W	N	c	C	q	K	5					D
	0107	892		a	r	t	W	N	c	S	q	K	5					
	0203	1000		a	r	t	W	N	c	C	q	K	5	0	95		1571	
93-04	9609	298		N	Q	N	Y	t	c	n	L	E	0	41	3			
	0012	361		N	Q	N	Y	t	c	n	L	E	0	69	9		13,368	
93-12	9306	296(191)		T	Q	N	Y	t	c	n	L	V	0	2	2	AZT		
	0011	343		a	Q	N	Y	t	c	n	L	V	2	91	84	HAART	<400	
93-32	0103	89		E	r	t	Y	t	c	C	q	m	6	0	0		AF462789	
93-60	9611	592		T	Q	N	Y	t	c	n	L	E	-1	0	0		Y clear	
	9906	418		T	Q	N	H	t	c	n	L	E	-2					
	0011	525		T	Q	N	H	t	c	n	L	E	-2				14,655	
	0107	491		T	Q	N	H	t	c	n	L	E	-2					
	0111	487		T	Q	N	H	t	c	n	L	E	-2	0	6			
95-95	0109	430		T	Q	N	#	t	c	n	L	V	0	12	6			
96-51	0204	766	PxxP	S	Q	N	Y	S	c	n	L	E	0	0	18			
98-33	0111	83	PxxP	Q	K	t	H	t	c	S	q	Q	4	44	0		33,957	

98-86	0110	594	PxxP	T	K	t	H	N	c	S	q	V	2	36	16		<400	
	0202			?	?	?	H	N	c	S	q	V	2			HAART	문맹렬	
99-36	0201	747		T	Q	S	Y	t	c	n	q	m	4			haart	유경준	
00-118	0101	No		T	Q	N	Y	t	c	n	L	V	0	0	0		양경원	
00-179	001226	657		Q	K	N	Y	N	c	S	q	m	2				이영숙	
	010824	460		K	K	N	Y	N	c	S	q	m	2					
	020424	186																
01-xx	0104	228		a	K	N	Y	t	c	S	L	m	2					
	0112	299		a	K	N	Y	t	c	S	q	m	4	0	6		홍수정	
01-LHS	0102	209		a	Q	N	Y	t	c	n	q	m	5	0	0		786,133	
	0112	715		a	Q	N	Y	t	c	n	q	m	5	10	0	HAART	<400	