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Research article

Genetic defects in the *nef* gene are associated with Korean Red Ginseng intake: monitoring of *nef* sequence polymorphisms over 20 years

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ABSTRACT

Background: The presence of gross deletions in the human immunodeficiency virus *nef* gene (gΔ*nef*) is associated with long-term nonprogression of infected patients. Here, we investigated how quickly genetic defects in the *nef* gene are associated with Korean Red Ginseng (KRG) intake in 10 long-term slow progressors.
Methods: This study was divided into three phases over a 20-yr period; baseline, KRG intake alone, and KRG plus highly active antiretroviral therapy (ART). *nef* gene amplicons were obtained using reverse transcription polymerase chain reaction (PCR) and nested PCR from 10 long-term slow progressors ($n = 1,396$), and nested PCR from 36 control patients ($n = 198$), and 28 ART patients ($n = 157$), and these were then sequenced. The proportion of gΔ*nef*, premature stop codons, and not in-frame insertion or deletion of a nucleotide was compared between three phases, control, and ART patients.
Results: The proportion of defective *nef* genes was significantly higher in on-KRG patients (15.6%) than in baseline (5.7%), control (5.6%), on-KRG plus ART phase (7.8%), and on-ART patients (6.6%; $p < 0.01$). Small in-frame deletions or insertions were significantly more frequent among patients treated with KRG alone compared with controls ($p < 0.01$). Significantly fewer instances of genetic defects were detected in samples taken during the KRG plus ART phase (7.8%; $p < 0.01$). The earliest defects detected were gΔ*nef* and small in-frame deletions after 7 mo and 67 mo of KRG intake, respectively.
Conclusion: KRG treatment might induce genetic defects in the *nef* gene. This report provides new insight into the importance of genetic defects in the pathogenesis of AIDS.
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1. Introduction

The introduction of highly active antiretroviral therapy (ART) from 1996 has dramatically reduced human immunodeficiency virus (HIV)-associated morbidity and mortality and transformed HIV infection into a chronic, manageable condition [1]. Even during sustained ART-mediated viral suppression, low-level viremia persistently occurs [2]. As a result of viremia, chronic immune activation and inflammation are accompanied by microbial translocation [3]. By contrast, there is no microbial translocation or immune activation in the well-adapted natural simian immune deficiency virus in hosts such as the sooty mangabeys (Cercocebus atys) [4], which is somewhat similar to the attenuated immune activation and slow progression observed in HIV-1-infected patients who have been treated with Korean Red Ginseng (KRG) for an extended period [5,6]. Interestingly, the saponin fraction of ginseng downregulates proinflammatory mediators in lipopolysaccharide-stimulated cells and protects mice against endotoxic shock [7–9] as well as anti-inflammatory activity [10]. In general, it is known that red ginseng has significantly higher biological effects and fewer side effects compared with fresh and white ginseng [11].

Among HIV-infected patients, many reports suggest that long-term nonprogressors (LTNPs) harbor grossly mutated copies of HIV *nef* more frequently than progressors [5,12–16], although there is some debate about the association between gross deletions in the *nef* gene (gΔ*nef*) and long-term nonprogression or slow progression [17,18]. In an Australian LTNP study group, three of 70 LTNPs were infected only with viruses containing *nef*-defective genomes, with no wild-type (WT) virus present [11,12]; all had viral loads of <200 copies/mL [15]. Some reports indicate that defects in the *nef*

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gene are more common during the late stages of disease [19]; however, the proportions of gΔ*nef* and small in-frame deletions (SDs) have never been investigated in relation to the effect of treatment in Western patient groups.

We had an opportunity to treat HIV-1-infected patients with KRG from 1991. Data analysis over a period of 60 mo revealed that KRG intake significantly delayed the decrease in CD4+ T cells compared with zidovudine monotherapy [20,21]. Using KRG, many patients maintained their CD4+ T cell counts for more than 10 yr without receiving ART. In addition, there was a significant inverse correlation found between KRG and the annual decrease of CD4+ T cells and between KRG and RNA copy number in our previous study [22]. Furthermore, we reported that, in addition to clinically beneficial effects, a high frequency of genetic defects, including gΔ*nef* [5,6,22–25], are associated with the long-term intake of KRG [26]. Consequently, we reported many long-term slow progressors (LTSPs) among a small Korean cohort of HIV-1 patients who have taken KRG for more than 10 yr [26]. Among the LTSPs, two patients have remained healthy for 26 yr after diagnosis in the absence of ART [26,27]. In the literature, Patient 87-05 might be the longest follow-up case in the absence of ART. Interestingly, all Korean LTSPs treated with KRG had viral loads of more than 3,000 copies/mL and revealed a high frequency of gΔ*nef* (18.8%) [5]. Moreover, recently we found that there is an association between taking KRG and gross deletions in the *pol* gene [28]. In addition, we reported for the first time that KRG intake has a synergistic antiviral effect in combination with ART *in vivo*, as well as acting to delete HIV-1 strains carrying resistant mutations [29].

Previously, we reported a possible association between KRG intake and gΔ*nef* [5,23,24] and the difference of the nature of gΔ*nef* in our LTSPs compared with Western patient groups [5]. Here, we investigated the time frame in which genetic defects in *nef* occur in response to KRG intake. We additively obtained sequence data at baseline (prior to KRG intake), after 1–2 yr of regular KRG intake and on KRG plus ART that were not included in the previous study [5]. In conclusion, these data show that genetic defects are associated with KRG intake and unlike SD, the detection of gΔ*nef* is decreased during ART. As the longest follow-up study of changes in the *nef* gene, spanning 20 yr, this report provides new insight into the importance of genetic defects and variation in the pathogenesis of AIDS.

2. Materials and methods

2.1. Patients

Ten patients included in this study who were infected with HIV subtype B were defined as LTSPs, with annual decreases in CD4+ T cells of $<20/\mu L$ over 10 yr [5]. The clinical characteristics of these patients, including changes in CD4+ T cell counts, RNA copy numbers, KRG therapy, and frequent genetic defects in *nef* and the 5′LTR/*gag*, have been described elsewhere [5,6]. However, the follow-up period of this study was extended to include the KRG plus ART period (Fig. 1). Control patients ($n = 36$) were selected from 216 available patients [30] using the following two criteria: first, patients that had not been exposed to KRG or any ART (zidovudine) at the sampling time point; and second, peripheral blood mononuclear cells (PBMCs) were used for HIV gene amplification. Twenty-eight ART only patients were also included for comparison with KRG plus ART group. This study was approved by the Institutional Review Board of the Asan Medical Center.

2.2. KRG intake

KRG capsules were manufactured from the roots of 6-yr-old fresh ginseng plants, *Panax ginseng* Meyer, harvested in the Republic of Korea by the Korea Ginseng Corporation, Seoul, Korea. KRG was made by steaming fresh ginseng at 90–100°C for 3 h and then drying at 50–80°C. One capsule contained 300-mg powder without any additives. There was an interruption to KRG intake of 4–5 mo after the first 6-mo pilot study.

2.3. Amplification of the nef gene

We used nested polymerase chain reaction (PCR) to amplify the proviral *nef* gene from PBMCs, as previously described [5,22,23]. Total RNA was extracted from 300 μL of serum samples using a QIAamp Ultra sense Viral RNA kit (Qiagen, Hilden, Germany), as previously described [30,31]. We compared sequence data at baseline (prior to KRG intake) and after 1–2 yr of regular KRG intake using reverse transcription-PCR analysis of data generated from serum, because the proportion of gΔ*nef* detectable in serum does not differ to that in PBMCs [32].

2.4. Definition of defective nef genes

A defective *nef* gene was defined as one with a premature stop codon or one lacking an initiation codon, and a gΔ*nef* was defined as a deletion of more than 15 nucleotides outside the variable region [5] as well as not in-frame insertion or deletion of a nucleotide. In this study, the amplicons revealing SDs, including that of the last cysteine, were considered intact.

2.5. Statistical analysis

Data are expressed as the mean ± two standard deviations (for continuous variables) or as counts and percentages (categorical variables). Proportions were compared between groups using the Chi-square or Fisher exact tests and logistic regression controlling for the subject effect (SPSS package version 12.0; SPSS Inc., Chicago, IL, USA). A p value < 0.05 was considered statistically significant.

2.6. Sequences

Genbank accession numbers for *nef* sequences are KM871217–KM871789, KM884898-940, and KU588425-KU588857.

3. Results

3.1. Effects of KRG during the follow-up period prior to ART administration

Ten LTSP patients received follow up for a mean of 199 ± 45 mo (16.6 yr; range: 164–314 mo) following HIV-1 diagnosis prior to ART (Table 1). During this period, KRG therapy was administered for over a mean 176 ± 42 mo and, thereafter, KRG plus ART was administered for 76 ± 44 mo (Fig. 1). The average total amounts of KRG administered per patient during these periods were 14,680 ± 5,940 g and 7,981 ± 6,050 g, respectively (Table 1). CD4+ T cells decreased from 455/uL to 169/uL for 173 ± 34 mo, corresponding to an annual decrease of 20.8 ± 14.8/μL.

3.2. Effects of KRG on the nef gene

Using samples from 10 LTSPs, we obtained 88 amplicons from sera at baseline, 962 amplicons (254 and 708 from sera and PBMCs, respectively) during KRG therapy only, and 346 amplicons from PBMCs during KRG plus ART. At baseline, five amplicons revealed defective *nef* genes (5.7%). For one patient (Patient 93-60), however, although a 7-bp deletion was detected at baseline, the virus had been previously exposed to KRG, because she was infected with

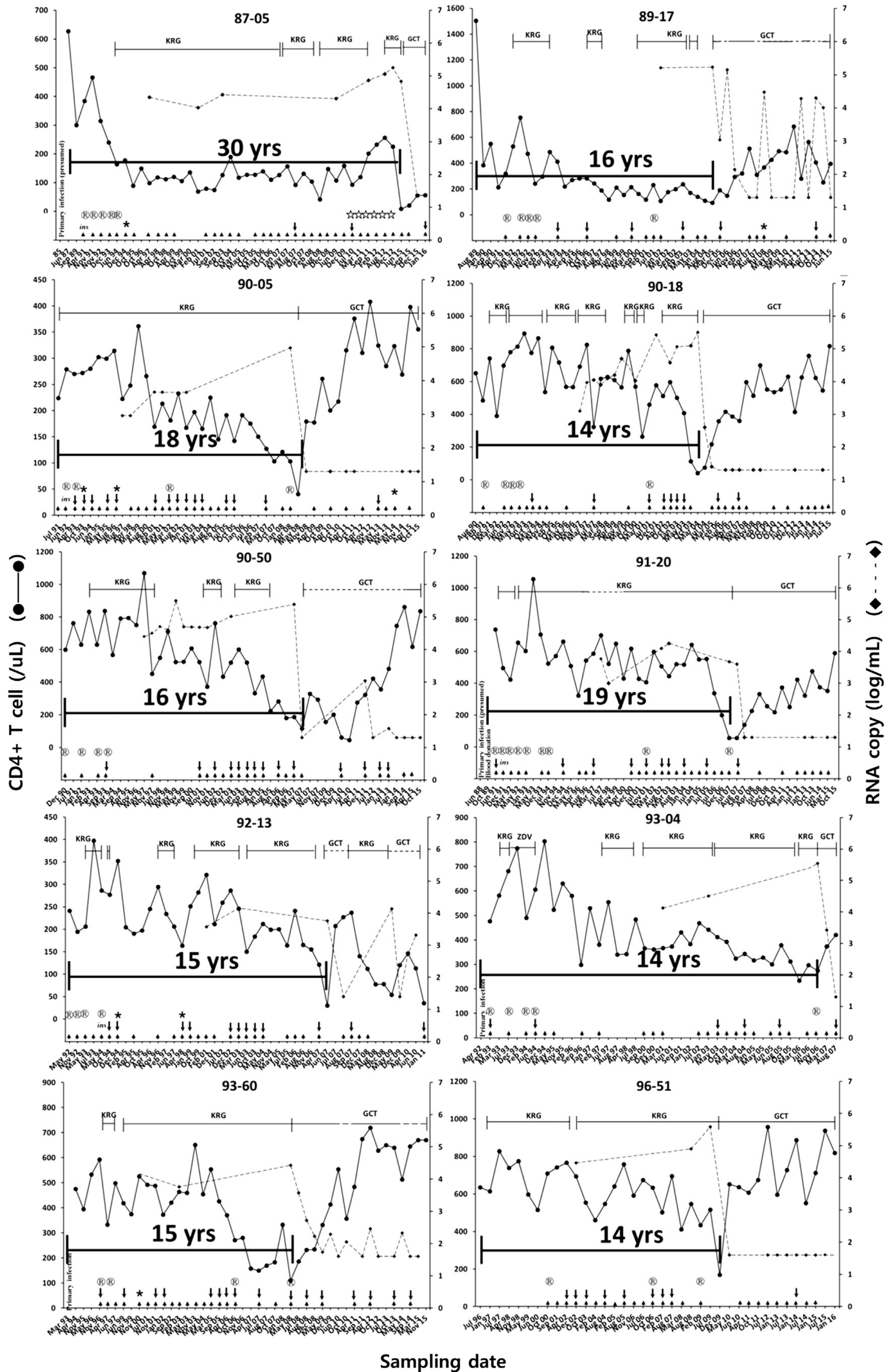

Fig. 1. Changes in the CD4+ T cell count, plasma viral load, and genetic defects according to Korean Red Ginseng (KRG) intake and highly active antiretroviral therapy (ART). The periods of KRG intake and ART, and duration of survival from diagnosis to initiation of ART are shown using a bar at the upper and middle parts, respectively. To our knowledge,

Table 1
Characteristics of 10 long-term slow progressors treated with Korean Red Ginseng (KRG)

Patient code[1)]	HIV-1 subtype	Genotyping for ART	Highest viral load (copy/mL) prior to ART	Amount of KRG supplied prior to & on ART(g)		Initiation of ART	Duration of ART (mo)
87-05	B	WT	69,600	21,258[2)]	900	Aug 2015	5
89-17	KSB	M184V[3)]	162,000	5,076	6,660	Mar 2005	123
90-05	KSB	WT	94,376	25,602	13,920	Jul 2008	87
90-18	KSB	WT	319,000	13,182	18,810	Apr 2004	135
90-50	KSB	WT	244,000	18,916	10,300	May 2007	101
91-20	KSB	WT	17,800	14,336	11,280	Aug 2007	98
92-13	KSB	WT	14,600	13,470	2,790	Jun 2007	45
93-04	KSB	K70R[3)]/WT	656,000	9,660	1,500	Nov 2006	9
93-60	KSB	WT	121,000	10,710	2,520	May 2008	90
96-51	KSB	WT	386,543	14,587	11,130	Dec 2009	66

Note. From "High frequency of grossly deleted *nef* genes in HIV-1 infected long-term slow progressors treated with Korean Red Ginseng," by Cho et al, 2006, *Current HIV Research*, 4, pp. 447–57. Copyright 2006, *Bentham Science Publishers*. Adapted with permission.
ART, highly active antiretroviral therapy; HIV, human immunodeficiency virus; KSB, Korean subclade of subtype B; WT, wild type.
[1)] First two digits before the hyphen in a patient code denotes the year of HIV-1 diagnosis.
[2)] Patient 87-05 took wild ginseng twice in 1983–1984.
[3)] Patients 89-17 and 93-04 revealed a single resistance mutation to lamivudine and zidovudine, respectively.

Table 2
Distribution of defective *nef* genes among the 10 long-term slow progressors

Patient code	Baseline		KRG only		KRG plus ART		Interruptions for ART
	No. of PCR	No. of defective	No. of PCR (from serum)[1)]	No. of defective	No. of PCR	No. of defective	
87-05	18	1^{ins}	157 (18)	$19 + 1^{pre}$	18	1	
89-17	16		63 (11)	11	29	$1+1^{pre}$	Presence
90-05	2		165 (40)	$26 + 2^{pre} + 2^{ins}$	28	$1+1^{pre}$	
90-18	11		77 (29)	12	65	2	
90-50	5		89 (34)	16	37	4	Presence
91-20	8	$1+1^{ins}$	71 (31)	11	59		
92-13	16		96 (44)	$13 + 2^{pre} + 1^{ins}$	18	2	Presence
93-04	5	1	96 (16)	4	14	1	
93-60	7	1	54 (17)	$18 + 1^{pre}$	20	12	Presence
96-51	ND	ND	94 (14)	11	58	1	
Total	88	5 (5.7%)	962 (254)	150 (15.6%)*	346	27(7.8%)**	

All *nef* amplicons at baseline and from controls were obtained by reverse transcription PCR and nested PCR, respectively.
* $p < 0.05$ compared with baseline.
** $p < 0.001$ compared with Korean Red Ginseng only.
ART, highly active antiretroviral therapy; ^{ins}, insertion of a nucleotide; KRG, Korean Red Ginseng; ND, not determined; PCR, polymerase chain reaction; ^{pre}, premature stop codon.
[1)] Number in parenthesis were obtained from sera by reverse transcription PCR.

HIV-1 in 1993 via her husband (Patient 89-17) who had taken KRG since 1991 (Table 2). From the untreated control group ($n = 36$), we obtained 198 amplicons from PBMC samples, eight (4.0%) and three (1.5%) of which were gΔ*nef* and premature stop codons (SC), respectively (5.6%). In the KRG-treated group, all 10 patients revealed gΔ*nef* and 150 (15.6%) of 962 amplicons were grossly defective, which was significantly higher compared with baseline ($p < 0.05$; Table 2) and control patients ($p < 0.001$; Fig. 2).

Focusing on how quickly gΔnef and other defects occur, we obtained 67 amplicons from the samples which were obtained 3–6 mo after treatment, none of which was a defective gene (0%). However, alterations gradually and significantly increased to 10.2% (6/59), 15.3% (13/85), and 17.4% (131/751) during 7–12 mo, 13–24 mo, and 24 mo after KRG intake, respectively ($p < 0.001$; Fig. 3). Even when the only gΔ*nef* was analyzed, there was a statistically significant difference to the baseline results.

The time for initial detection of all *nef* gene defects, including SC was 7 mo. However, the actual median time is likely to be even earlier. The proportion of gΔ*nef* in this study was slightly lower than that in another study population treated with KRG for 3 yr without interruption [5,23]. Patient 90-50, who took a higher dose of KRG (9 g/d) since February 1993, was the first in which a gΔ*nef* was detected in September 1993. Thus, the first occurrence of gΔ*nef* was after 7 mo of KRG intake, which is the earliest detection of gΔ*nef* to date. gΔ*nef* was not detected within the first 6 mo of KRG intake even when other patients were included. However, there were no differences in SCs and not in-frame insertions between the three phases.

3.3. Effect of ART on nef

Detection of genetic defects including gΔ*nef* was significantly decreased during the time when patients received KRG plus ART (7.8%) compared with the KRG-only period (15.6%, $p < 0.001$; Table 2), although the average amount of KRG/mo was higher on KRG plus ART (7,981/76 mo; 105 g) than in KRG only (14,680 g/176 mo; 84 g). Even excluding amplicons containing SCs and not in-frame insertions, the results remain statistically significant (14.8% vs. 7.4%, $p < 0.001$). However, four patients (89-17, 90-50, 92-13, and 93-60) with poor compliance or several interruptions for ART

Patient 87-05 is the longest follow-up case in the absence of ART in the literature. Patient 91-20 had a history of admission under suspicion of typhoid fever and blood donation on October 28, 1989. His blood recipients were infected with human immunodeficiency virus-1. The downward arrow (↓), ▲, ®, dotted line in the highest position (–), and asterisk (*) symbols at the base of the figure denote gross deletion, wild-type amplicon only, reverse transcription PCR, poor compliance, and premature stop codon, respectively. GCT, Korean Red Ginseng based combination therapy with ART; PCR, polymerase chain reaction.

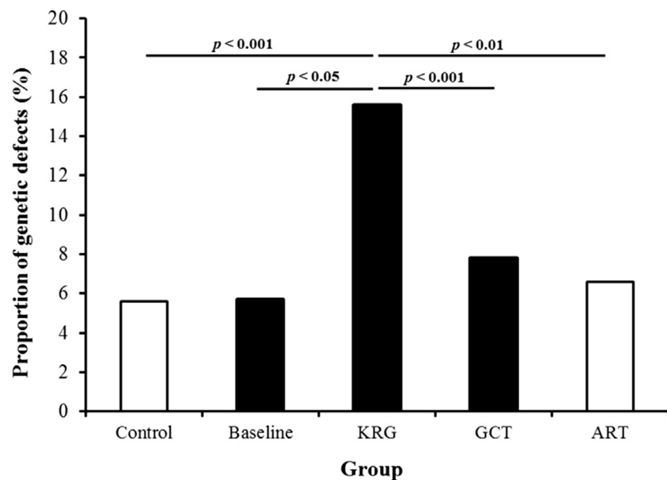

Fig. 2. Comparison of the proportion of genetic defects such as gross deletion in the *nef* gene, not in-frame insertion or deletion, and premature stop codon. The proportion of genetic defects was significantly higher during Korean Red Ginseng (KRG) intake than in controls or at baseline and decreased significantly during GCT [KRG plus antiretroviral therapy (ART)]. In 28 ART patients, the numbers of gross deletions in the *nef* gene and stop codons were six (3.6%) and five (3.0%) of 165 amplicons, respectively.

(revealing viremia $<$ 20 copies/mL) revealed unusually high proportions of gΔ*nef* (19/104; 18.3%) even on KRG plus ART. In contrast, the remaining six patients with good compliance (no viremia $<$ 20 copies/mL) revealed a very low proportion of gΔ*nef* (6/242; 2.5%, $p < 0.001$; Table 2). These data suggest that detection of gΔ*nef* might be used as an indicator for poor compliance for ART on KRG plus ART.

Analysis of the *nef* gene revealed SCs in six of 708 amplicons (0.9%) on KRG alone and 0.6% (2/346) on KRG plus ART (Table 3). These proportions were significantly lower than 3.6% (6/168) in 28 ART only patients ($p < 0.05$).

3.4. Small deletions ranging from 6 bp to 18 bp and insertions of 6 bp to 9 bp

Patient 87-05 was found to carry a virus with a 6-bp deletion in *nef* at the baseline, although the patient reported two periods of wild ginseng intake in 1983–1984 and 2002. We obtained 10 amplicons from the *nef* gene from the earliest sample from this

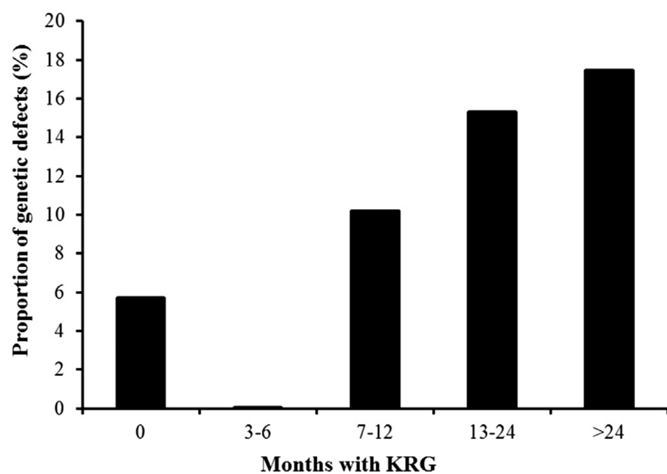

Fig. 3. The proportion of genetic defects in the *nef* gene depends on the duration of Korean Red Ginseng (KRG) intake ($p < 0.001$). There was no defective gene 3–6 mo after KRG treatment (0/68). Genetic defects increased significantly after 12 mo compared with baseline ($p < 0.05$).

Table 3
Comparison of the proportion of polymerase chain reaction amplicons containing premature stop codons among four genes

Genes	Control (%)	KRG (%)	KRG plus ART	p^{**}
nef	3/198 (1.5)	6/708 (0.9)*	2/346 (0.6)*	
Vif [33]	1/106 (0.9)	3/275 (1.1)*	12/157 (7.6)*	$<$0.001
Pol [31]	2/90 (2.2)	1/82 (1.2)	19/214 (8.9)*	$<$0.05
Gag [25]	0/30 (0)	8/107 (7.5)*	20/147 (13.6)*	

The study on *vif* gene [33] was done for the same patients with this study.
* $p < 0.01$.
** $p < 0.01$ between KRG and KRG plus ART.
ART, antiretroviral therapy; KRG, Korean Red Ginseng.

patient (April 1991 prior to KRG intake). Of these, five were WT, two contained a 6-bp deletion of nucleotides encoding amino acids (AAs) 8–9, two had a 9-bp deletion of nucleotides encoding AAs 9–11, and one had WT (Fig. S1). After about 20 yr, only a smaller deletion of 3 bp (encoding AA 10) was detected. We hypothesize that these SDs could be related to the long-term slow progression of this patient who remained healthy for >28 yr in the absence of ART. Various other gene alterations were identified in viral material from this patient, including a SC in December 1994, gΔ*nef* in August 2007 and July 2010, and a deletion of 2 bp (nucleotides 2,977 and 2,978 in HIV-1 NL4-3) in the *pol* gene, although we did not classify these SDs as defective genes ($n = 63$; Fig. S1). In addition, G-to-A hypermutations in the *nef* gene were observed in 22 sequences amplified from this patient after December 2009 (Fig. 1). The deletion of *nef* AAs 9–11 is very rare, although it has previously been reported in GenBank sequence AY265085 (Cameroon CRF02-AG).

In addition, Patient 90-18 had 30 *nef* amplicons with two different 6-bp deletions; encoding AAs 50–51 67 mo after baseline and AAs 47–48 132 mo after baseline. All 19 samples obtained during KRG intake contained both WT and deleted amplicons. The most common deletion in *nef* is of nucleotides encoding AAs 60–61 and this was reported in two patients by Alexander et al [34]. These deletions occurred on KRG intake and were similar to the 9-bp deletion in the *vif* gene in samples from the same patient [33]. Samples from Patient 91-20 and Patient 92-13 demonstrated 9-bp (AAs 151–153; $n = 1$) and 18-bp (AAs 151–156; $n = 1$) deletion after 10 yr and 14 yr of KRG intake in November 2001 and April 2007, respectively. An amplicon from Patient 90-05 (January 2003) revealed a novel insertion of 3 bp between the codons for AAs 25 and 26 and a further 15 amplicons (July 2005) with 6-bp insertions between AAs 27 and 28 (Fig. S1). Amplicons with a deletion of the last cysteine in *nef* were identified in three patients (Patient 89-17, Patient 92-13, and Patient 93-60; Fig. S1). SDs appeared in both serum and PBMCs regardless of the type of specimen as shown for gΔ*nef*.

In summary, except for Patient 87-05, there were no such deletions or insertions at baseline (0/70; $p = 0.068$) or in the control group (0/159; $p < 0.01$), compared with KRG alone (47/962).

4. Discussion

Our previous reports have shown that long-term intake of KRG slows depletion of CD4 T cells in HIV-1 infected patients irrespective of HLA Class I alleles [35]. In addition to clinical relevance, sequence data have indicated a strong association between KRG treatment and gΔ*nef* in regard to dosage and duration [5,24,25]. However, previous data did not clarify when and how early the gΔ*nef* can be induced by KRG treatment. In this study, we found that the gΔ*nef* was first observed after 7 mo of KRG intake. gΔ*nef* was not detected within the first 6 mo of KRG intake even when other patients were included. However, we still do not know the mechanism of gΔ*nef* by KRG treatment. The reasons are as follows:

firstly, ginseng contains many active components and we applied whole ginseng for patients. Secondly, the occurrence of gΔ*nef* is <30% in the level of PCR amplicons, although it was detected 100% in patient levels over the long term. Thirdly, it is well documented in literature that ginseng modulates nearly all kinds of immune cells, although the main direction is via cell mediated immunity and innate immunity [9,10] than humoral immunity. Fourthly, there is no similar report on any therapeutic agents, including medicinal food, that deletes or attenuates the "microorganisms or invader gene," although antibiotics or antiviral drugs develop specific resistance mutations or insertions.

With respect to the possible mechanism of KRG, a few reports also support our data. For example, polyacetyleneginsenside-Ro, xylanase, and quinqueginsin of ginseng have been shown previously to have inhibitory effects on HIV-1 reverse transcriptase [36–38]. It is possible that these inhibitory effects on RT might decrease its fidelity and thereby result in a high frequency of genetic defects.

Our data showed that the proportion of detected gΔ*nef* is significantly decreased in KRG plus ART. Effective ART reduces the virus concentration from about 10,000 copies/mL to <20 copies/mL. Deleted genes comprise a minor portion compared with intact genes *in vivo*. Thus, ART significantly decreases the chance of detecting deleted genes. This is therefore a kind of "marginal dilution effect" rather than actual inhibition of occurrence of gΔ*nef* [25].

In addition to the SD mentioned above, we found similar SDs (6–15 bp) in 11 further Korean patients among the 216 analyzed [30]. In detail, nine of these were in amplicons from patients taking KRG, whereas another two were in patients not taking KRG. Among the nine patients, four patients carried viruses with SDs (two nucleotides encoding AAs 10–11 and two of AAs 8–12) compared with baseline samples (Fig. S2). The position of the SD was very similar to that in amplicons from Patient 87-05 (AAs 9–11). The remaining five patients had a WT *nef* sequence at baseline with SD appearing after at least 37 mo of KRG intake; deletion of codons for AAs 47–48 in one patient after 37 mo (Patient KYJa; Fig. S2). This occurrence was earlier than after 76 mo in Patient 90-18 and after 12 yr in Patient 92-13 in the *vif* gene [33]. This may indicate that the *nef* gene is more variable than *vif* [30,33]. Interestingly, one patient (Patient JHiS) was infected with WT virus via her husband (Patient CHR). Her husband took more KRG (4,620 g for 8 yr) than her (2,820 g for 8 yr), and a SD was observed earlier in the husband, suggesting a dose-relationship between KRG intake and SD occurrence. In addition, four out of nine patients revealed a deletion of the cysteine prior to the stop codon (Fig. S2). By contrast, SD was detected in two patients in the absence of KRG intake. In conclusion, our data suggest the possibility of associations between KRG intake and SD, although SD occurred relatively rarely and later than gΔ*nef*.

The report by Alexander et al [34] supports the findings of the current study. Although there is no consensus on the association of SDs with prognosis, an elite suppressor (ES10-53) revealed two SDs in *nef* [39], and it is particularly interesting that the position of these deletions was the same or adjacent to those of five patients in this study.

Regarding the immunological mechanism underlying the occurrence of gΔ*nef*, we would like to put a high value on the potentiation of cytotoxic T lymphocyte activity. gΔ*nef* and SD might result indirectly from immune modulation toward a Th1 cytokine profile, an anti-inflammatory response [7–10], in addition to viral suppression [29].

In addition to this immunological pressure on proviral DNA within the host chromosome, many cellular factors could be involved in provirus latency. For example, it is well known that chromatin remodeling enzymes like histone deacetylases (HDACs) recruited to the HIV promoter play an important role in HIV latency. HDAC inhibitors might lead to the activation of HIV in latently infected cells and result in the fragmentation of proviral DNA. Recently, Compound K, which is a major metabolite of ginseng saponin, has been found to act as an HDAC inhibitor [40].

In conclusion, this report provides new insights into the importance of genetic defects and variations in the *nef* gene in the pathogenesis of AIDS.

Conflicts of interest

All contributing authors declare no conflicts of interest.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jgr.2016.02.005.

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