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Divergent and dynamic activity of endogenous retroviruses in burn patients and their inflammatory potential

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Abstract

Genes constitute ~3% of the human genome, whereas human endogenous retroviruses (HERVs) represent ~8%. We examined post-burn HERV expression in patients' blood cells, and the inflammatory potentials of the burn-associated HERVs were evaluated. Buffy coat cells, collected at various time points from 11 patients, were screened for the expression of eight HERV families, and we identified their divergent expression profiles depending on patient, HERV, and time point. The population of expressed HERV sequences was patient-specific, suggesting HERVs' inherent genomic polymorphisms and/or differential expression potentials depending on characteristics of patients and courses of injury response. Some HERVs were shared among the patients, while the others were divergent. Interestingly, one burn-associated HERV *gag* gene from a patient's genome induced IL-6, IL-1β, Ptgs-2, and iNOS. These findings demonstrate that injury stressors initiate divergent HERV responses depending on patient, HERV, and disease course and implicate HERVs as genetic elements contributing to polymorphic injury pathophysiology.

Keywords

HERV polymorphism; burn patient; divergent injury response

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Conflict of Interest

The authors declare that they have no conflict of interest.

Introduction

The stress signals originating from burn injury sites are often transmitted to distant organs through various layers of both characterized and uncharacterized pathways, leading to divergent and often unpredictable clinical manifestations such as inflammatory disorder and organ failure (Fayazov et al., 2009; Kallinen et al., 2012). The mechanisms underlying the complex and polymorphic network of post-burn pathologic events have been investigated primarily by studying the relationship between burn-incited phenotypes and altered functions of genes, focusing on differential expression profiles and non-synonymous single nucleotide polymorphisms (SNPs) (Barber et al., 2004; Barber et al., 2006). Although substantial progress has been made in understanding the basics of local and distant response to burn injury, the vast majority of the multifactorial characteristics of the disease courses and clinical outcomes occurring in a heterogeneous population of burn patients are far from being fully grasped.

Human endogenous retroviruses (HERVs) occupy ~8 % of the human genome while the entire set of protein coding genes consists of only ~3 % (Lander et al., 2001; Venter et al., 2001). HERVs are reported to participate in a range of disease processes such as degeneration of oligodendrocytes, type-I diabetes mellitus, rheumatoid arthritis, and breast cancer (Conrad et al., 1997; Contreras-Galindo et al., 2008; Frank et al., 2005; Freimanis et al., 2010). In addition, the envelope (*env*) polypeptides of certain murine endogenous retroviruses (ERVs) are capable of inducing pro-inflammatory cytokines (*e.g.*, IL-6) in macrophages (Lee et al., 2011). Burn-elicited stress signals have been found to differentially alter the expression of murine ERVs, some of which retain intact coding potentials for virion assembly, in a tissue/cell type- and time after injury-specific manner (Cho et al., 2008; Kwon et al., 2009; Lee et al., 2008), unpublished data). The ERVs, which are activated in response to burn-incited stress signals, may exert their biologic activity via their gene products and/or replication/infection (Boller et al., 2008; Holder et al., 2012; Weis et al., 2007). Alternatively, ERVs, which are integrated into genes, may affect their neighboring genes through their transcription regulatory activity and post-transcriptional modifications, including alternative splicing that leads to the generation of fusion transcripts (Feuchter-Murthy et al., 1993; Medstrand et al., 2001; Ting et al., 1992).

The human population, regardless of genetic background, is presumed to share a substantial number of HERV loci in their genomes; however, at the same time, it is anticipated that each individual has a unique genomic HERV profile. We postulate that the polymorphic HERV profiles in the genomes of a heterogeneous population of burn patients are closely linked to the divergent and often unpredictable disease courses and outcomes. In this study, post-burn changes in the HERV expression profiles were examined in a heterogeneous patient population, and pathologic properties of the gene products of the burn-associated HERVs were examined.

Materials and Methods

Patient population and blood collection

This study has been reviewed and approved by the Institutional Review Boards Administration of the University of California, Davis and University of Michigan, Ann Arbor in accordance with the common rule and any other governing regulations. Participants or the next of kin, caretakers, or guardians on the behalf of the minors/children provide their written informed consent to participate in this study. Subjects enrolled in this study had a minimum of a 30% total body surface area burn. Detailed information regarding the patients and schedules for the blood sample collection is summarized in Table 1. Approximately 4–8 ml of blood samples were collected at several time points up to 270 days post-admission.

Semi-quantitative RT-PCR analyses of HERV expression

Buffy coat was isolated from each blood sample by centrifugation at 2,000 x*g* for 10 minutes at room temperature. Total RNA was isolated from the buffy coat using the RNeasy Mini kit (Qiagen, Valencia, CA) with modifications, including treatment with TRIzol (Invitrogen, Carlsbad, CA) and DNase I (to remove any genomic DNA contamination). cDNA was synthesized using 100 ng of total RNA from each sample, Sensiscript reverse transcriptase (Qiagen), RNase inhibitor (Promega, Madison, WI) and an oligo-dT primer (5′-GGC CAC GCG TCG ACT AGT ACT TTT TTT TTT TTT TTT T- 3′). The absence of genomic DNA contamination in the cDNA preparations was verified using the control samples without reverse transcriptase treatment. The primer sets, which were used to amplify the 3′ long terminal repeat (LTR) regions of eight different HERV families, are listed in Table 2. β-actin was amplified as a normalization control using the primer set: 5′-CCA ACT GGG ACG ACA TGG AG-3′ and 5′-GTA GAT GGG CAC AGT GTG GG-3′. Densitometric quantitation was performed for the individual HERV amplicons using the Kodak MI system (Carestream Health, Rochester, NY). The intensity of each HERV amplicon was normalized with the matching β-actin.

Cloning and sequencing

A total of 344 HERV amplicons (from patient-1, patient-2, patient-4, and patient-11) were purified using the QIAquick Gel Extraction kit (Qiagen) and then cloned into the pGEM-T Easy vector (Promega). Three clones were picked for each amplicon, and plasmid DNAs were prepared using the QIAprep Miniprep kit (Qiagen) for sequencing analysis. Sequencing was performed at Functional Biosciences (Madison, WI). DNA sequences were analyzed using the EditSeq and MegAlign programs (DNASTAR, Madison, WI).

Multiple alignment and phylogenetic analyses of expressed HERV sequences within each HERV family

A total of 1,026 3′ LTR region sequences were obtained from the 344 HERV amplicons. To evaluate whether the expressed HERV sequences are shared among the four patients (patient-1, patient-2, patient-4, and patient-11), the LTR region sequences were subjected to alignment analyses within each HERV family using the ClustalW protocol, and phylogenetic trees were generated using the MEGA4 program (Tamura et al., 2007).

In silico mapping of HERV loci

Among the 137 and 202 unique 3′ LTR region sequences which were identified from patient-1 and 2, respectively, only 37 sequences were shared by both patients. The reference human genome database (Build 37.1) from the National Center for Biotechnology Information (NCBI) was surveyed for putative HERVs which share greater than 98 % identity using each unique 3′ LTR region sequence as a mining probe and the Advanced Blast program. The percent identity was reduced to 95 % or 90 % step-wise if no hits were retrieved with the 98 % identity threshold. The regions, which span 12 Kb upstream and downstream from the individual LTR hits, were surveyed to identify putative HERV loci. For each putative HERV locus, the coding potentials for three genes (*gag*, *pol*, and *env*) were examined using the SeqBuilder program (DNASTAR). The open reading frames, which encode greater than 100 amino acids, were recorded and the others were denoted as defective.

Cloning of *gag* polypeptide coding sequences from a patient's genomic DNA

The *gag* polypeptide coding regions of two different HERVs were amplified from patient-1's genomic DNA by a two-step PCR protocol using a combination of two primer sets for each HERV to obtain locus-specificity (primer sequences are listed in Table 2). First, the 5′ LTR-*gag* regions were amplified (30 cycles) using a set of primers that span the 5′-proviral junction to the end of the *gag* coding sequence. During the second round of PCR (20 cycles), the specific *gag* coding regions (start to end) were amplified from the 5′ LTR-*gag* amplicon from the first PCR, followed by cloning into the pGEM-T Easy vector (Promega) and subcloning into the pcDNA4/HisMax expression vector (Invitrogen). All constructs were sequenced to confirm the inserts.

Real-time RT-PCR measurement of inflammatory mediators in RAW264.7 cells

RAW264.7 cells were transfected with individual pcDNA4/HisMax expression constructs (two *gag* coding sequences, one *gag* in reverse coding orientation, and vector only). Transfected cells were harvested at day 1 to examine changes in the expression (mRNA) of a set of six inflammatory mediators by real-time RT-PCR (primer sequences for each mediator are listed in Table 2). Total RNA was isolated using the RNeasy Mini kit (Qiagen), and real-time RT-PCR was performed using the QuantiTect RT kit (Qiagen) and Brilliant III SYBR green QPCR master mix in the Mx3005P cycler (Agilent Technologies, Santa Clara, CA).

Statistical analysis

Statistical analysis was performed using a one-way ANOVA and Tukey's HSD test, and statistical significance was determined as $*P < 0.05$ and $**P < 0.01$.

Results

Divergent and dynamic HERV expression profiles among burn patients

The blood samples of the 11 patients (Table 1), which were collected at various post-admission time points, were subjected to semi-quantitative RT-PCR analyses to determine

whether burn-elicited stress signals and accompanying clinical courses alter the expression of 12 HERV families (Table 2). Within each patient, there were dynamic changes in the expression patterns of the individual HERV families in a time after injury-dependent manner (Figure 1). The patient-specific temporal HERV expression profile may be directly linked to the inherent genomic HERV polymorphisms among patients and/or differential expression depending on age, gender, clinical courses, and/or injury severity. Some HERV families (*e.g.*, HERV-E, HERV-K(HML-1) [HERV-K1], RRHERV-I) were expressed only in certain patients, but not in others. In another analysis for individual patients, at each time point, the post-injury fold-changes in all HERV amplicons derived from the 12 families were compiled in order to examine accumulative temporal changes in HERV expression (Figure 2). There were substantial differences in the accumulative levels of HERV expression over the various post-injury time points in certain patients (*e.g.*, patient-1, patient-2) compared to the others (*e.g.*, patient-4, patient-8, patient-10). Since only a limited number of patients were enrolled in this study, it may not be practical to correlate the HERV expression data with any specific disease courses (*e.g.*, septic episodes) and/or treatment protocols (*e.g.*, transfusion, surgery).

Prevalence of uncommon and patient-specific expressed HERV sequences

The entire set of HERV RT-PCR amplicons from four patients (patient-1, patient-2, patient-4, and patient-11) was subjected to sequence analyses. One to three 3′ long terminal repeat (LTR) sequences were obtained from each visible amplicon: 211 3′ LTR sequences from 67 amplicons in patient-1; 276 sequences from 92 amplicons in patient-2; 297 sequences from 99 amplicons in patient-4; and 242 sequences from 86 amplicons in patient-11. For each patient, the population of HERV LTR sequences derived from the entire set of amplicons was sorted by multiple alignment and phylogenetic analysis to identify unique LTR sequences within each HERV family as well as among all the HERV families combined. There were 137, 202, 154, and 159 unique sequences from patient-1, patient-2, patient-4, and patient-11, respectively, when all the HERV amplicons were combined. It needs to be noted that certain HERV families were not expressed in all four patients. Among the 3′ LTR sequences of HERV-H (both H1 and H2 amplicons) (23 from patient-1, 27 from patient-2, 37 from patient-4, and 23 from patient-11), only two were shared by all four patients, and the vast majority were unique for each patient (Figure 3-panel c). Similarly, none of the HERV-K(HML-2) (HERV-K2) LTR sequences (15 from patient-1, 21 from patient-2, 16 from patient-4, and 17 from patient-11) were shared among the four patients and only two sequences, one from the HERV-K(HML-4) (HERV-K4) family and the other HERV-K(HML-5) (HERV-K5) family, were found in all four patients (Figure 3-panel d). Among the LTR sequences from all HERV families, only 10 sequences were shared by all four patients while each patient had a number of unique sequences ranging from 64 (patient-1) to 94 (patient-11) (Figure 3-center panel). These findings suggest that there is a high prevalence of uncommon expressed HERV sequences among the four patients examined in this study.

HERV family-specific differential population diversity of expressed 3′ LTR sequences among the patients

In a separate study, the relatedness of the expressed HERV sequences among the four patients was measured for individual HERV families. In Figure 4, the degree of relatedness of the 3′ LTR sequences of each HERV family among the four patients (patient-1, patient-2, patient-4, and patient-11) is indicated by a spoke on the graph, which represents each patient in comparison to the other three or two patients. The distance from the center of the circle indicates a decrease in the relatedness between the patients being compared. The 3′ LTR sequences of three HERV families (FRD, H(1), and K5) were relatively divergent among the patients whereas the HERV-R LTR sequences were highly homologous. It was interesting to note that the 3′ LTR sequences of HERV-FRD and HERV-H(1) from patient-11 were most distantly related to the other three patients. The data obtained from this study indicates that the populations of the post-burn expressed 3′ LTR sequences of certain HERV families are more individual patient-specific than the others. It is likely that these differential population diversities are associated with the patients' genomic HERV profiles as well as the evolutionary phylogenic characteristics of the individual HERV families.

In silico mapping and characterization of burn-associated HERVs

Using the entire set of the uniquely expressed HERV LTR sequences obtained from patient-1 and patient-2 as probes, the reference human genome database from the National Center for Biotechnology Information (NCBI) was surveyed to identify putative HERVs which retain substantial coding potentials of at least 500 amino acids for three retroviral polypeptides (*gag*, *pol*, and/or *env*). The identity threshold was set to "at least 98 %" for both LTR hits during the survey. A total of 18 putative HERV loci, which are capable of coding at least one of the three polypeptides with a minimum coding potential of 500 amino acids (Table 3), were mapped; 15 of them were identified using the probes derived from both patients. The sizes of the putative HERV proviral sequences ranged from 6,373 to 10,222 nucleotides. The lengths of putative coding sequences for the individual polypeptides were somewhat variable: *gag* polypeptide (647 to 756 amino acids), *pol* polypeptide (954 to 1,014 amino acids), and *env* polypeptide (538 to 699 amino acids). Only one putative HERV locus was determined to retain coding potentials for all three polypeptides (Table 3).

Differential induction of inflammatory mediators by *gag* polypeptides of two burn-associated HERVs

It has been reported that a sub-genomic murine ERV, which retains a full coding potential only for the *gag* polypeptide, participates in inflammatory disorders in mice (Cho et al., 2002; Jolicoeur, 1991). In addition, our previous study demonstrated that burn-elicited stress signals rapidly and transiently induce expression of a sub-genomic murine ERV in the liver of mice (Cho et al., 2002). In this study, the inflammatory potentials of the *gag* polypeptides of two burn-associated HERVs were evaluated. Two putative HERV loci (HERV-K109 [chromosome 6] and HERV-K115 [chromosome 8]), which were mapped on the NCBI reference genome with the 3′ LTR sequences from patient-1 and patient-2, were selected to clone the putative *gag* polypeptide coding sequences. The sizes of the two *gag* polypeptides on the reference loci were 666 (HERV-K109) and 648 (HERV-K115) amino acids (Figure

5). A two-step PCR scheme was developed to target the specific *gag* coding sequences of the individual HERV loci using the genomic DNA of patient-1. Sequence analyses revealed that the two *gag* coding regions isolated from patient-1's genomic DNA harbor significant mismatches (*e.g.*, missense mutation, C-terminus variation) in comparison to the respective reference loci (Figure 5). The *gag* coding region obtained from the putative HERV-K109 locus was 666 amino acids (same size as reference) and the second *gag* coding sequence (from the putative HERV-K115 locus) was 545 amino acids (648 amino acids for the reference locus). This finding confirms the existence of HERV polymorphisms between the genomes of NCBI reference and patient-1.

To evaluate the inflammatory potentials of the two HERV *gag* polypeptides, which were derived from the putative HERV-K109 and HERV-K115 loci of patient-1's genome, individual *gag* polypeptides were overexpressed in RAW264.7 macrophage cells followed by the measurement of changes in mRNA production of six inflammatory mediators: IL-6, IL-1β, iNOS, Ptgs-2 (COX-2), TNF-α, and ICAM-1 (Figure 6). The expression of IL-6, IL-1β, iNOS, and Ptgs-2 was substantially increased following over-expression of the *gag* polypeptide of HERV-K109$_{Pt1}$, which was presumed to be derived from the putative HERV-K109 locus on chromosome 6 of the genomic DNA of patient-1. In contrast, over-expression of the *gag* polypeptide of HERV-K115$_{Pt1,}$ presumed to be cloned from the putative HERV-K115 locus (chromosome 8), resulted in an increase of only IL-1β expression (~ 5 fold compared to over 40 fold by HERV-K115$_{Pt1}$ *gag* polypeptide). Interestingly, the expression of ICAM-1 was reduced by the *gag* polypeptide of HERV-K109$_{Pt1}$. The difference in inflammatory properties between the *gag* polypeptides of HERV-K109$_{Pt1}$ and HERV-K115$_{Pt1}$ may be explained by: 1) C-terminus polymorphisms, in particular, a C-terminus truncation of 121 amino acids in the HERV-K115$_{Pt1}$ *gag* polypeptide and 2) 25 mismatched amino acids distributed throughout the polypeptides. It needs to be noted that the C-terminus 121 amino acid region includes various functional motifs, such as CCHC-type 1, CCHC-type 2, and a glutamine-rich region (Figure 5) (Bayer et al., 1995; Dorfman et al., 1993).

Discussion

The complex network of post-burn pathogenesis has been investigated primarily by focusing on common genetic polymorphisms and expression profiles of select genes which are reported to be responsible for a host of pathologic phenotypes, such as inflammation, cytotoxicity, and apoptosis (Barber et al., 2006; Feezor et al., 2005; Schwacha et al., 2005). However, a comprehensive knowledge in regard to the proteins, genetic elements, and cells, which control the divergent and often unpredictable pathologic episodes occurring in burn patients, has not yet been formulated. Genomic sequences dedicated to conventional protein-coding genes only comprise ~3 % of the human genome while HERVs and related elements make up ~8 % (Lander et al., 2001; Venter et al., 2001). Our recent findings that burn-incited stressors differentially activate ERVs in mice and that some murine ERV gene products harbor pro-inflammatory potential led us to investigate HERV responses in burn patients (Kwon et al., 2009; Lee et al., 2007; Lee et al., 2011; Lee et al., 2008). It is possible that HERVs and other human genomic elements, such as small interspersed nuclear elements (SINEs) and long interspersed nuclear elements (LINEs), could also participate in burn-elicited pathologic events.

The patient-specific and highly polymorphic post-burn HERV response patterns observed in this study could be attributed to several factors. These patterns may be directly linked to a series of specific pathologic episodes and/or treatment regimens which are unique for individual patients with divergent genetic backgrounds. On the other hand, it is likely that the diversity in genomic HERV profiles among the patient population, which account for uncommon genetic variations, contributed to the divergent HERV responses and variable responses to injury. Specifically, patient-specific genomic HERVs may play a role, at least in part, in the variable and uncommon post-burn pathologic episodes. The expression of certain HERV loci (*e.g.*, HERV-K109$_{Pt1}$), which reside only on the genomes of certain patients, may be differentially induced post-burn in conjunction with their unique transcription regulatory profiles and inherent epigenetic status (Chiu et al., 2010; Conley and Jordan, 2012; Rebollo et al., 2012).

The gene products of the HERVs, which were induced in response to burn-elicited stress signals, may participate in patient-specific pathogenesis. In fact, the finding from this study that the *gag* polypeptide of putative HERV-K109$_{Pt1}$ retains substantial potential to induce pro-inflammatory mediators in comparison to the other *gag* polypeptide (HERV-K115$_{Pt1}$) implies that uncommon variations in genomic HERV profiles may be directly linked to the divergent post-burn pathologic courses observed among patient populations. Further studies focusing on the biological significance of the 25 mismatched amino acids between the two *gag* polypeptides as well as a C-terminus truncation of 121 amino acids in the *gag* polypeptide of HERV-K115$_{Pt1}$ will shed fresh insights into the impacts of genomes' uncommon variations on divergent post-burn pathogeneses.

Conclusions

The findings from this study provide some evidence that certain HERVs contribute to divergent, often unpredictable, disease courses of a heterogeneous population of burn patients. In addition, the uncommon variant loci of the genomes of the human population, such as HERV-K109$_{pt1}$, may serve as critical genomic markers for the development of tailored treatment regimens for different individuals.

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Abbreviations

ERVs	endogenous retroviruses
HERVs	human endogenous retroviruses
HERV-K1	HERV-K(HML-1)
HERV-K2	HERV-K(HML-2)

HERV-K4	HERV-K(HML-4)
HERV-K5	HERV-K(HML-5)
HERV-K6	HERV-K(HML-6)
LTRs	long terminal repeats
NCBI	national center for biotechnology information
SINEs	short interspersed nuclear elements
LINEs	long interspersed nuclear elements
SNPs	single nucleotide polymorphisms
Pt	patient

References

Barber RC, et al. TLR4 and TNF-alpha polymorphisms are associated with an increased risk for severe sepsis following burn injury. J Med Genet. 2004; 41:808–13. [PubMed: 15520404]

Barber RC, et al. Innate immunity SNPs are associated with risk for severe sepsis after burn injury. Clin Med Res. 2006; 4:250–5. [PubMed: 17210974]

Bayer P, et al. Structural studies of HIV-1 Tat protein. J Mol Biol. 1995; 247:529–35. [PubMed: 7723010]

Boller K, et al. Human endogenous retrovirus HERV-K113 is capable of producing intact viral particles. J Gen Virol. 2008; 89:567–72. [PubMed: 18198388]

Chiu S, et al. Post-injury stress signals alter epigenetic profile of cytosine methylation in the proviral genome of endogenous retroviruses. Exp Mol Pathol. 2010; 89:291–300. [PubMed: 20609362]

Cho K, et al. Induction of murine AIDS virus-related sequences after burn injury. J Surg Res. 2002; 104:53–62. [PubMed: 11971678]

Cho K, et al. Endogenous retroviruses in systemic response to stress signals. Shock. 2008; 30:105–16. [PubMed: 18317406]

Conley AB, Jordan IK. Endogenous Retroviruses and the Epigenome. Viruses: Essential Agents of Life. 2012:309–323.

Conrad B, et al. A human endogenous retroviral superantigen as candidate autoimmune gene in type I diabetes. Cell. 1997; 90:303–13. [PubMed: 9244304]

Contreras-Galindo R, et al. Human endogenous retrovirus K (HML-2) elements in the plasma of people with lymphoma and breast cancer. J Virol. 2008; 82:9329–36. [PubMed: 18632860]

Dorfman T, et al. Mapping of functionally important residues of a cysteine-histidine box in the human immunodeficiency virus type 1 nucleocapsid protein. J Virol. 1993; 67:6159–69. [PubMed: 8371356]

Fayazov AD, et al. Disorders of the immune system in severely burned patients. Ann Burns Fire Disasters. 2009; 22:121–30. [PubMed: 21991166]

Feezor RJ, et al. Functional genomics and gene expression profiling in sepsis: beyond class prediction. Clin Infect Dis. 2005; 41(Suppl 7):S427–35. [PubMed: 16237642]

Feuchter-Murthy AE, et al. Splicing of a human endogenous retrovirus to a novel phospholipase A2 related gene. Nucleic Acids Res. 1993; 21:135–43. [PubMed: 8382789]

Frank O, et al. Human endogenous retrovirus expression profiles in samples from brains of patients with schizophrenia and bipolar disorders. J Virol. 2005; 79:10890–901. [PubMed: 16103141]

Freimanis G, et al. A role for human endogenous retrovirus-K (HML-2) in rheumatoid arthritis: investigating mechanisms of pathogenesis. Clin Exp Immunol. 2010; 160:340–7. [PubMed: 20345981]

Holder BS, et al. Syncytin 1 in the human placenta. Placenta. 2012; 33:460–6. [PubMed: 22381536]

Jolicoeur P. Murine acquired immunodeficiency syndrome (MAIDS): an animal model to study the AIDS pathogenesis. FASEB J. 1991; 5:2398–405. [PubMed: 2065888]

Kallinen O, et al. Multiple organ failure as a cause of death in patients with severe burns. J Burn Care Res. 2012; 33:206–11. [PubMed: 21979843]

Kwon DN, et al. Cloning and characterization of endogenous retroviruses associated with postinjury stress signals in lymphoid tissues. Shock. 2009; 32:80–8. [PubMed: 18948849]

Lander ES, et al. Initial sequencing and analysis of the human genome. Nature. 2001; 409:860–921. [PubMed: 11237011]

Lee YK, et al. Genome-wide changes in expression profile of murine endogenous retroviruses (MuERVs) in distant organs after burn injury. BMC Genomics. 2007; 8:440. [PubMed: 18045489]

Lee YK, et al. Tropism, cytotoxicity, and inflammatory properties of two envelope genes of murine leukemia virus type-endogenous retroviruses of C57BL/6J mice. Mediators Inflamm. 2011; 2011:509604. [PubMed: 21772664]

Lee YK, et al. Genome-wide expression profiles of endogenous retroviruses in lymphoid tissues and their biological properties. Virology. 2008; 373:263–73. [PubMed: 18187179]

Medstrand P, et al. Long terminal repeats are used as alternative promoters for the endothelin B receptor and apolipoprotein C-I genes in humans. J Biol Chem. 2001; 276:1896–903. [PubMed: 11054415]

Rebollo R, et al. Epigenetic interplay between mouse endogenous retroviruses and host genes. Genome Biol. 2012; 13:R89. [PubMed: 23034137]

Schwacha MG, et al. Genetic variability in the immune-inflammatory response after major burn injury. Shock. 2005; 23:123–8. [PubMed: 15665726]

Tamura K, et al. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol Biol Evol. 2007; 24:1596–9. [PubMed: 17488738]

Ting CN, et al. Endogenous retroviral sequences are required for tissue-specific expression of a human salivary amylase gene. Genes Dev. 1992; 6:1457–65. [PubMed: 1379564]

Venter JC, et al. The sequence of the human genome. Science. 2001; 291:1304–51. [PubMed: 11181995]

Weis S, et al. Reduced expression of human endogenous retrovirus (HERV)-W GAG protein in the cingulate gyrus and hippocampus in schizophrenia, bipolar disorder, and depression. J Neural Transm. 2007; 114:645–55. [PubMed: 17219017]

Highlights

- Divergent and dynamic post-burn HERV expression profiles in blood cells of patients
- Patient-specific population of post-burn expressed HERV sequences
- Induction of IL-6, IL-1β, COX-2, and iNOS by *gag* gene of a burn-associated HERV
- Implication of HERVs as genetic elements contributing to divergent injury pathology

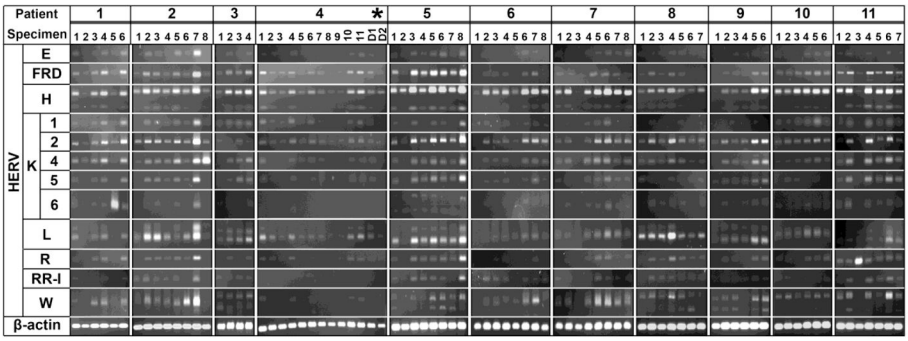

Figure 1. Post-burn alterations in HERV expression: patient-, HERV-, and time after injury-specific patterns

Post-burn changes in the expression of 12 different HERV families were analyzed by semi-quantitative RT-PCR. The post-burn HERV expression profiles were specific for patient, HERV family, and time point (Please refer to Table 1 for detailed time point information). *Blood samples from the two daughters of patient-4 (D1 and D2) were included as no-injury controls. β-actin serves as a normalization control. RR-I (RRHERV-I).

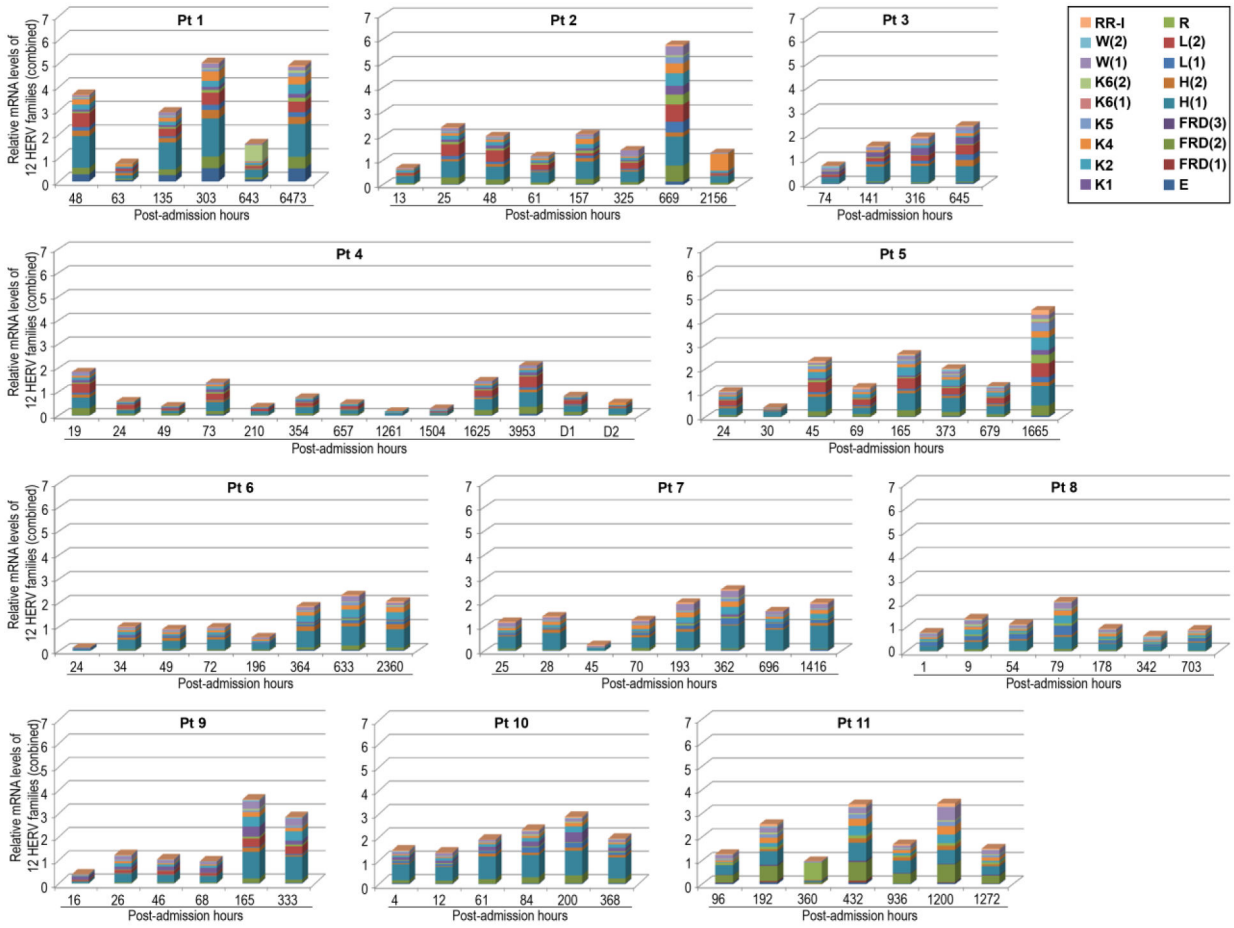

Figure 2. Dynamic and patient-specific changes in the overall HERV expression at different post-burn time points

The post-burn changes in the levels of the individual amplicons from 12 HERV families were combined at each time point (represented as a single mosaic bar) for each patient. Five HERV families (FRD, H, L, K(HML-6) [K6] and W) had multiple amplicons, which are indicated by sequential numbers in parenthesis in the legend. There were dynamic and patient-specific changes in the overall expression of HERVs at various post-burn time points. Pt (patient); RR-I (RRHERV-I).

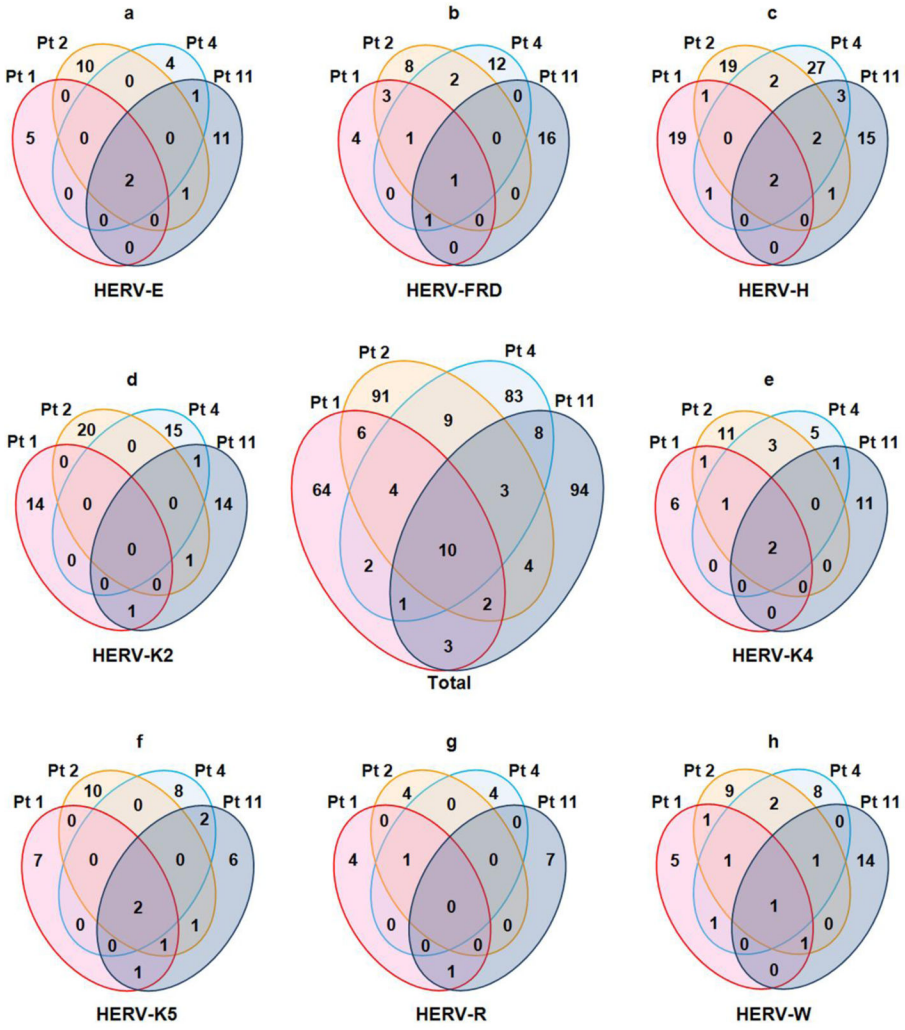

Figure 3. High degree of polymorphisms in post-burn expressed HERVs among patients

The HERV 3′ LTR sequences, which were isolated from RT-PCR amplicons of HERV-E, HERV-FRD, HERV-H, HERV-K2, HERV-K4, HERV-K5, HERV-R, and HERV-W from each patient, were compared among four patients (patient-1, patient-2, patient-4, and patient-11) by multiple alignment analyses. A Venn diagram, which shows the numbers of shared and unique HERV LTR sequences, was drawn for each HERV family to show the polymorphisms of expressed HERV sequences among the four patients. The center Venn diagram (labeled “Total”) combines the results from all eight HERV families. The profiles of post-burn expressed HERVs were highly polymorphic among the four patients in all eight HERV families.

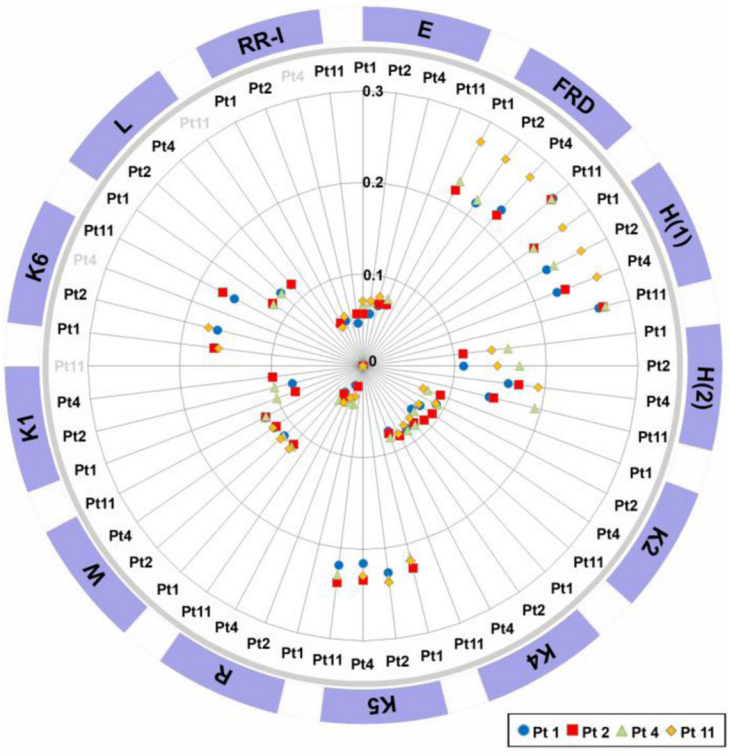

Figure 4. HERV family-specific population diversity of post-burn expressed HERVs among four patients

Within each HERV family, the diversity of post-burn expressed HERV populations among four patients was measured. Each patient's HERV population was compared to the HERV populations of the other three or two patients within each HERV family. Individual patients are identified with various symbols. As the distance from the center increases, the relatedness of the indicated HERV populations to the reference HERV population decreases (*e.g.*, Pt1 [patient-1] vs. Pt2, Pt4, and Pt11). Within the HERV-FRD, HERV-H(1), and HERV-K5 families, the expressed HERV populations were relatively diverse among the four patients in comparison to the other families, such as HERV-E, HERV-K2, and HERV-K4. HERV-H(1) and HERV-H(2) were derived from two RT-PCR amplicons of HERV-H. HERV-K1, HERV-K6, HERV-L and RRHERV-I were examined only in three patient groups. RR-I (RRHERV-I).

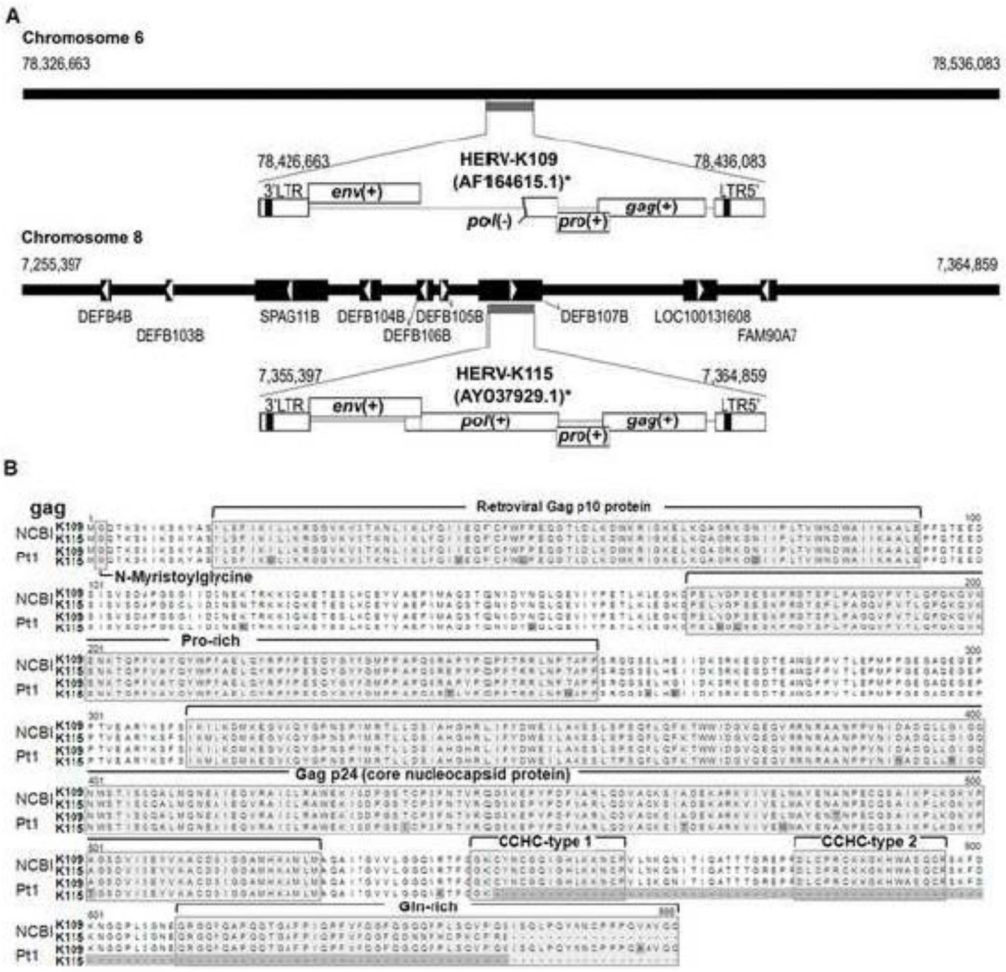

Figure 5. Genomic map of two burn-associated HERV loci and comparison of their putative *gag* polypeptide sequences

A. HERV-K109 and HERV-K115 were mapped *in silico* on the NCBI human reference genome using the post-burn expressed HERV LTR sequences as a mining probe. The proviral structures of putative HERV-K109 and HERV-K115, which were mapped on chromosome 6 and 8, respectively, were drawn with their neighboring annotated genes. Horizontal thick line (chromosome); black box with arrow (gene); +/− (intact/defective open reading frame) **B.** The putative *gag* polypeptide sequences of HERV-K109 and HERV-K115 from the NCBI human reference genome and patient-1 genome were compared. Mismatched amino acid positions and a C-terminus truncation are highlighted in grey. Processed peptides and key motifs are also indicated. NCBI (reference human genome); Pt1 (patient-1).

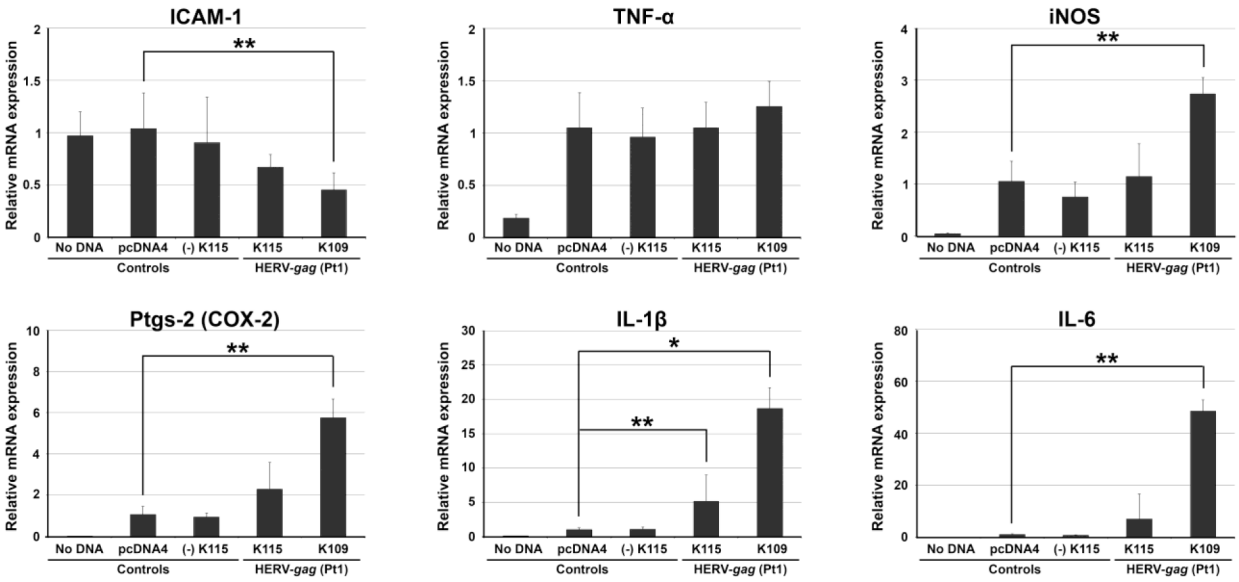

Figure 6. HERV-*gag* isoform-specific induction of inflammatory mediators in macrophage cells

The two *gag* isoforms from putative HERV-K109$_{Pt1}$ and HERV-K115$_{Pt1}$, which were isolated from patient-1's genomic DNA, were examined for their effects on the expression of inflammatory mediators upon overexpression in RAW264.7 cells. Overexpression of HERV-K109$_{Pt1}$-*gag* substantially induced various inflammatory mediators, such as IL-1β and IL-6, iNOS, and Ptgs-2 (COX-2) while only a slight increase in IL-1β expression was observed with HERV-K115$_{Pt1}$-*gag*. There are three different negative controls: No DNA, pcDNA4 (empty pcDNA4/HisMax plasmid), and (−)K115$_{Pt1}$-*gag* (reverse oriented HERV-K115$_{Pt1}$-*gag* insert in pcDNA4). K109$_{Pt1}$-*gag* (HERV-K109$_{Pt1}$-*gag*); K115$_{Pt1}$-*gag* (HERV-K115$_{Pt1}$-*gag*). *P < 0.05; **P < 0.01; error bar (standard deviation).

Table 1

Patient demographics and sample collection time points.

Patients	Age	Sex	Race	Sample collection: Post-burn time points (hour)										
				1	2	3	4	5	6	7	8	9	10	11
Patient-1	6	F	C	48	63	135	303	643	6473					
Patient-2	13	M	C	13	25	48	61	157	325	669	2156			
Patient-3	20	M	C	74	141	316	645							
Patient-4	57	M	C	19	24	49	73	210	354	657	1261	1504	1625	3953
Patient-4:D1	N/A	F	N/A											
Patient-4:D2	N/A	F	N/A											
Patient-5	44	M	C	24	30	45	69	165	373	679	1665			
Patient-6	3	M	H	24	34	49	72	196	364	633	2360			
Patient-7	2	M	H	25	28	45	70	193	362	696	1416			
Patient-8	17	F	H	1	9	54	79	178	342	703				
Patient-9	12	M	H	16	26	46	68	165	333					
Patient-10	5	F	H	4	12	61	84	200	368					
Patient-11	53	F	C	96	192	360	432	936	1200	1272				

F (female); M (male); C (Caucasian); H (Hispanic).

* Blood samples from the two daughters (D1 and D2) of patient-4 were obtained as a no-burn control.

Table 2

(top) primers for HERVs, (middle) primers for *gag* genes, and (bottom) primers for inflammatory mediators

HERV family		Primer name	Sequence	Reference
HERV	E	HERV-E(F) HERV-E(R)	CACTTCTCCTGTTGTCCTT TACAACTCTAAGGGGTCT	M10976 and AB062274
	FRD	HERV-Frd(F) HERV-Frd(R)	CAG**G/T**CTCCCAA**C/T**AGATAGA AGAGCAGGA**G/A**TGAAAGGAA	AC004022
	H	HERV-H(F) HERV-H(R)	CTGATGA**C/T**ATTCCACCA AGACCACCAAACAGG**C/G**TT	D11078, AJ289709, AJ289711, M18048
	L	HERV-L(F) HERV-L(R)	GATTG**G/T**AT**G/T**GAAGGATGC CTGAGTCTGAGTCCCAAAGC	X89211
	R	HERV-R(F) HERV-R(R)	AGGAAAAACAAGTAAAGGG CCGTGACAGGTTTTACAA	M12410
	K1	HERV-K(HML1)(F) HERV-K(HML1)(R)	CTGCTCTCCATTATCTCAAGT AAGGAATGAGAAAAGACAGT	Repbase version 8.2.0 consensus sequences
	K2	HERV-K(HML2)(F) HERV-K(HML2)(R)	GTCATCACCACTCCCTAATCT GGAAAAGAAAAAGACACAGAG	AF074086, AF164609, AF164610
	K4	HERV-K(HML4)(F) HERV-K(HML4)(R)	TGTGGGCGAAGGATTACC AATGTCCCTTCAGCACCT	AF020092
	K5	HERV-K(HML5)(F) HERV-K(HML5)(R)	GCAGAGGGCAAGGAGTAG GCGATGGATGAAAGGAGTA	Repbase version 8.2.0 consensus sequences
	K6	HERV-K(HML6)(F) HERV-K(HML6)(R)	CAGAATGTGGGCAAACTC TGAACAAAGGAGGACGAA	AF079797
	W	HERV-W(F) HERV-W(R)	AGAGCACAGCAGGAGGGA GGGGTCCTTGCTCACAGA	AY101582, AY101583, AY101584 AY101585
	RRI	RRHERV-I(F) RRHERV-I(R)	TATCTCTCCCTTTCCCAG AGGAATCAGAGAGACC**A/GG/A**TG	M64936

HERV	Region	Primer name	Sequence	Reference
HERV-K109 (AF164615.1)	LTR-gag	6D01_LTR-1A 3D02_Sgag-2A	CAGTCTCAGGTGTTTGGATCTTCCAC CGCG*gcggccgc*CTACTGCTGCACTGCCGCTTGT	NT_007299.13 (16546496-16555953)
	gag	3D02_Sgag-1A 3D02_Sgag-2A	CGCG*gcggccgc*ATGGGGCAAACTAAAAGTAAAG CGCG*gcggccgc*CTACTGCTGCACTGCCGCTTGT	
HERV-K115 (AY037929.1)	LTR-gag	8D01_LTR-1A 3D02_Lgag-2A	TGATCAATATAAAAGGTGTAGGGGTGG CGCG*gcggccgc*GCTTATTCCCTGAAACACTTGGGAC	NT_023736.17 (7345381-7354859)
	gag	3D02_Lgag-1B 3D02_Lgag-2A	CGCG*gcggccgc*GCTAGGGTGATAATGGGGCAAAC CGCG*gcggccgc*GCTTATTCCCTGAAACACTTGGGAC	

Cytokines	Primer name	Sequence	Reference
Ptgs2	COX2_1B COX2_2A	ACACAGTGCACTACATCCTGAC ATCATCTCTACCTGAGTGTC	NM_011198.3
ICAM1	ICAM1_ 1A ICAM1_ 2A	AGCTGTTTGAGCTGAGCGAGA CTGTCGAACTCCTCAGTCA	NM_010493.2
Il1b	Il1b_ 1A Il1b_ 2A	GACAGTGATGAGAATGACCTG GAACTCTGCAGACTCAAACTCCA	NM_008361.3
Il6	Il6_1E Il6_2G	GCCTTCCCTACTTCACAAGTCC CACTAGGTTTGCCGAGTAGATCTC	NM_031168.1
Nos2	Nos2_1B Nos2_2B	ACAAGCTGCATGTGACATCGA CAGAGCCTGAAGTCATGTTTGC	NM_010927.3
Tnf	Tnf_1A Tnf_2A	GCATGATCCGCGACGTGGAA AGATCCATGCCGTTGGCCAG	NM_013693.2

Cytokines	Primer name	Sequence	Reference
b-actin	b-actin-1A b-actin-2A	CCAACTGGGACGTGGAA GTAGATGGGCACAGTGTGGG	NM_007393.3 NM_001101.3

bold font (degenerate nucleotides); *italic* font (NotI restriction enzyme site)

Table 3

Putative HERVs mined from the NCBI reference genome using the burn-associated HERVs as a probe

Among the putative HERVs mined from this study, only the ones which retain at least one 500 amino acid coding potential for *gag*, *pol*, or *env* polypeptides are listed.

	HERV locus	Contig	Chr	+/−	Chr location		Size	*gag*			*pol*			*env*		
					from	to		start	end	size (a.a.)	start	end	size (a.a.)	start	end	size (a.a.)
Pt 1	01-H-6A-02_2D01 (HERV-H/env62)*	NT_005403.17	2	−	166564060	166572708	8649	1227	1874	215			(−)	6255	8009	584
Pt 1 and Pt 2	12-K2-2A-04_1D02	NT_032977.9	1	+	75842771	75849143	6373			(−)			(−)	3685	5451	588
	12-K2-2A-04_1D01 (HERV-K50A)	NT_004487.19	1	−	155596457	155605636	9180	1112	1648	178	4131	6434	767	6492	8231	579
	12-K2-2A-04_3D01	NT_005612.16	3	−	112693124	112702282	9159	1104	3188	694	3870	6014	714	6478	7728	416
	12-K2-2A-04_3D02 (HERV-K50B)	NT_005612.16	3	−	185230336	185239436	9101	1032	3038	668	3800	6709	969			(−)
	12-K2-2A-04_5D01 (JN675037.1)*	NT_023133.13	5	−	156084717	156093896	9180	1112	3112	666	3879	6923	1014			(−)
	12-K2-2A-04_6D01 (HERV-K109)	NT_007299.13	6	−	78426663	78436083	9421	1104	3104	666**	3871	4455	195	6412	8508	698
	12-K2-2A-04_7D01 (HML-2.HOM)*	NT_007819.17	7	−	4622057	4631528	9472	1865	2401	178	4632	7502	956	7204	9303	699
	12-K2-2A-04_7D02 (HML-2.HOM)*	NT_007819.17	7	−	4630560	4640030	9471	1111	2205	364	3877	6747	956	7203	9302	699
	12-K2-2A-04_8D01 (HERV-K115)	NT_023736.17	8	−	7355397	7364859	9463	1104	3047	647**	3870	6740	956	6442	8541	699
	12-K2-2A-04_11D01 (JN675064.1)*	NT_033899.8	11	+	101565794	101575259	9466	926	1648	240	3879	6743	954	7198	9183	661
	12-K2-2A-04_12D01 (JN675068.1)*	NT_029419.12	12	−	58721242	58730698	9457	1113	3113	666			(−)	6440	8536	698
	12-K2-2A-04_22D01 (JN675087.1)*	NT_011519.10	22	+	18926187	18935361	9175	1112	3112	666			(−)	6491	8248	585
	12-K2-4A-01_3D01 (JN675021.1)*	NT_005612.16	3	+	101360737	101369859	9123	1113	3116	667			(−)			(−)
	12-R-3A-05_7D01 (ERV3-1)	NT_007933.15	7	−	64450702	64460320	9619			(−)			(−)	7893	9707	604
	12-W-5A-01_7D01 (ERVW-1)	NT_007933.15	7	−	92097285	92107506	10222			(−)	4564	4974	136	7812	9428	538
Pt 2	02-E-7A-01-17D01 (AB062274.1)*	NT_010799.15	17	−	26557545	26566365	8821	1462	2655	397	2689	4371	560	6264	6923	219
	02-K2-7A-09-1D01 (JN675013.1)*	NT_004487.19	1	+	160660575	160669807	9233	1113	1874	253	4188	5777	529	6544	8226	560

Pt (patient); Chr (chromosome); +/− (strand orientation); underline (putative full coding potential);

* HERV references with an incomplete match to the corresponding putative HERV loci identified in this study;

** two *gag* polypeptides which were subjected to functional analysis.