A Human Endogenous Retrovirus K dUTPase Triggers a T_H1, T_H17 Cytokine Response: Does It Have a Role in Psoriasis?

Maria-Eugenia Ariza[1,2] and Marshall V. Williams[2,3,4]

Psoriasis is a chronic inflammatory immune disease of the skin characterized by a complex interplay between multiple risk genes and their interactions with environmental factors. Recent haplotype analyses have suggested that deoxyuridine triphosphate nucleotidohydrolase (dUTPase) encoded by a human endogenous retrovirus K (HERV-K) may be a candidate gene for the psoriasis susceptibility 1 locus. However, no functional studies have been conducted to determine the role of HERV-K dUTPase in psoriasis. For this purpose, we constructed an HERV-K dUTPase wild-type sequence, as well as specific mutations reflecting the genotype characteristic of high- and low-risk haplotypes, purified the recombinant proteins, and evaluated whether they could modulate innate and/or adaptive immune responses. In this study, we demonstrate that wild-type and mutant HERV-K dUTPase proteins induce the activation of NF-κB through Toll-like receptor 2, independent of enzymatic activity. Proteome array studies revealed that treatment of human primary cells with wild-type and mutant HERV-K dUTPase proteins triggered the secretion of T_H1 and T_H17 cytokines involved in the formation of psoriatic plaques, including IL-12p40, IL-23, IL-17, tumor necrosis factor-α, IL-8, and CCL20, in dendritic/Langerhans-like cells and to a lesser extent in keratinocytes. These data support HERV-K dUTPase as a potential contributor to psoriasis pathophysiology.

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INTRODUCTION

Psoriasis is an inherited chronic inflammatory disease of the skin that affects 2–3% of the world's population. Epidemiological and genetic data suggest that psoriasis development is a complex process influenced by several risk genes, by environmental factors such as viral infections, stress, and trauma, and by the interplay between various cells involved in innate and adaptive immunity, which are capable of altering the skin microenvironment through cytokine and chemokine effector molecules, ultimately resulting in the differentiation of resident skin cells to form psoriatic lesions (Lowes *et al.*, 2007; Nickoloff, 2007; Nickoloff *et al.*, 2007; Nestle *et al.*, 2009).

Of the nine chromosomal loci that have been statistically associated with psoriasis through classical genome-wide linkage analysis (Bowcock, 2005; Bowcock and Krueger, 2005), the psoriasis susceptibility 1 (PSORS1) locus, located in the major histocompatibility complex on chromosome 6p21, has been identified as the strongest genetic determinant of psoriasis, accounting for 35–50% of the heritability of the disease. Genetic analyses have suggested that HLA-Cw6 is the PSORS1 susceptibility gene (Nair *et al.*, 2000, 2006; Elder, 2006; Elder *et al.*, 2010), and, although several theories have been proposed to explain how HLA-Cw6 may trigger the cytokine/chemokine cascade that is characteristic of psoriasis (Nickoloff *et al.*, 2007), molecular mechanistic studies demonstrating it have been a challenge and it still remains unclear and poorly understood.

Human endogenous retrovirus (HERV) sequences constitute 7–8% of the human genome composition (Rebora, 2005). Most HERVs have become defective because of recombination events and by the accumulation of nonsense mutations ensuring that no protein or viable virus particles are produced. In contrast, members of the human endogenous retrovirus K (HERV-K) family comprise less than 1% of the HERV sequences found in the human genome, but they are transcriptionally active. Although the replication competent virus has not been demonstrated *in vivo*, several HERV-K

[1]*Department of Environmental Health Sciences, Arnold School of Public Health, University of South Carolina, Columbia, South Carolina, USA;* [2]*Department of Molecular Virology, Immunology, and Medical Genetics, The Ohio State University Medical Center, Columbus, Ohio, USA;* [3]*Comprehensive Cancer Center, The Ohio State University Medical Center, Columbus, Ohio, USA and* [4]*Institute for Behavioral Medicine Research, The Ohio State University Medical Center, Columbus, Ohio, USA*

Correspondence: Maria-Eugenia Ariza, Department of Molecular Virology, Immunology, and Medical Genetics, The Ohio State University Medical Center, Columbus, Ohio 43210, USA. E-mail: maria.ariza@osumc.edu

Abbreviations: AA, amino acid; DM, double mutation; dUTPase, deoxyuridine triphosphate nucleotidohydrolase; hDC, dendritic/Langerhans-like cell; HERV-K dUTPase, human endogenous retrovirus K-encoded dUTPase; HR, high risk; LR, low risk; PSORS1, psoriasis susceptibility locus 1; TLR2, Toll-like receptor 2

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proviruses, such as K113 and K115, have been shown to contain open reading frames for all genes (Barbulescu *et al.*, 1999; Mayer *et al.*, 1999; Bannert and Kurth, 2004; Kim *et al.*, 2004). Perhaps more importantly is the feature that some HERV-K proviruses exhibit an insertional polymorphism (Turner *et al.*, 2001; Belshaw *et al.*, 2005; Moyes *et al.*, 2005, 2007). There are several reports linking endogenous retroviruses with numerous autoimmune diseases including psoriasis (Bannert and Kurth, 2004; Colmegna and Garry, 2006; Guilhou and Moles, 2008). However, none of these studies have demonstrated a causal relationship between virus-encoded proteins and psoriasis pathogenesis.

It has also been reported that the target region of the PSORS1 harbors a fragment of HERV-K, which encodes for a deoxyuridine triphosphate nucleotidohydrolase (dUTPase; Foerster *et al.*, 2005). dUTPases represent a family of metalloenzymes that catalyze the hydrolysis of dUTP to dUMP and pyrophosphate; until recently, their only known function was preventing dUTP incorporation into DNA by DNA polymerases (McGeoch, 1990; Mayer and Meese, 2003). Using microarray expression profiling and functional assays, we recently demonstrated that the monomeric dUTPase encoded by the Epstein–Barr virus possesses, functions in innate immunity partly because of the activation of Toll-like receptor (TLR) 2 and the subsequent modulation of downstream genes involved in type I IFN (IFNα/β) and cytokine/chemokine receptor signaling pathways (Glaser *et al.*, 2006; Waldman *et al.*, 2008; Ariza *et al.*, 2009, 2011). As psoriasis is a chronic inflammatory disease and the PSORS1 locus may encode a HERV-K dUTPase it is logical to hypothesize that the HERV-K dUTPase may possess immunomodulatory functions similar to that of the Epstein–Barr virus-encoded dUTPase. Furthermore, there is accumulating evidence supporting a potential role of TLRs in psoriasis. Baker *et al.* (2003) reported that, although TLR1, 2, and 5 are constitutively expressed primarily by keratinocytes located in the basal layer of the epidermis in normal skin, TLR2 was expressed primarily in keratinocytes located in the upper epidermis of the lesional skin of psoriasis patients. However, the biological relevance of the altered TLR expression in psoriasis remains unknown.

Our hypothesis is that the HERV-K dUTPase protein triggers the secretion of proinflammatory T_H1/T_H17 cytokines and it may contribute to psoriasis pathogenesis by modulating host innate and adaptive immune responses leading to immune dysregulation. To address this hypothesis, we constructed a wild-type DNA sequence of the HERV-K dUTPase, as well as sequences containing specific mutations in the gene that reflect the low- and high-risk haplotypes identified by Foerster *et al.* (2005); the purified proteins from these genes were used to determine whether they could modulate innate and adaptive immune responses leading to the activation of cytokine/chemokine cascades. In this study, we demonstrate that the purified HERV-K dUTPase activates NF-κB in a transgenic human embryonic kidney 293 (HEK293) cell line stably expressing TLR2, but not in wild-type HEK293 cells. We also demonstrate that treatment of normal human dendritic/Langerhans-like cells (hDCs), and to a less extent keratinocytes, with the purified proteins results in the increased production of cytokines/chemokines IL-1β, IL-6, Il-8, IL-10, IL-12p40, IL-17, IL-23, transforming growth factor-α, tumor necrosis factor-α, RANTES, CCL20, and IFN-γ, known to be involved in the formation of psoriatic plaques. Finally, we demonstrate that the ability of HERV-K dUTPase to modulate the expression of these cytokines/chemokines is dependent in part upon the presence or absence of specific AA residues between P141 and D171.

RESULTS

Cloning of the *HERV-K dUTPase* gene and mutant construction

To test the hypothesis that the HERV-K dUTPase protein may be a trigger of psoriasis and/or may contribute to psoriasis pathogenesis by modulating host innate and adaptive immune responses leading to immune dysregulation, the gene encoding HERV-K dUTPase was PCR amplified from human genomic DNA and cloned into the pTrcHis plasmid for protein expression. A comparison of DNA sequences from the low- and high-risk haplotypes, reported by Foerster *et al.* (2005), with the wild-type sequence used in this study, and initially reported by Harris *et al.* (1997), demonstrates that there is extensive variation in the C-terminal end of these proteins. Therefore, in an effort to demonstrate whether the C-terminal end of the HERV-K dUTPase might be responsible for promoting immune dysregulation, we also created a series of point or C-terminal deletion mutations (Figure 1) that were verified by DNA sequence analysis. Recombinant proteins were purified as described in Materials and Methods (Figure 2) and screened for dUTPase activity. None of the recombinant HERV-K proteins possessed dUTPase activity when compared with human dUTPase, which was used as a positive control, even after extended incubation (24 vs. 1 hour for control) and using a 10-fold higher amount of protein (0.4 vs. 0.04 μg; data not shown).

The HERV-K dUTPase activates TLR2

To explore the possibility that HERV-K dUTPase could be modulating innate inflammatory immune responses through TLR2, we investigated whether the HERV-K dUTPase recombinant proteins could trigger NF-κB activation using the transgenic HEK293 cell line stably expressing TLR2, as described previously (Ariza *et al.*, 2009). Briefly, TLR2-expressing HEK293 cells were transiently transfected with the NF-κB luciferase reporter gene and transfection control pRL-TK vectors, followed by treatment with various HERV-K dUTPase recombinant proteins ($10\,\mu g\,ml^{-1}$), zymosan (a natural ligand for TLR2), or vehicle control. Human dUTPase protein was used as a negative control. As demonstrated in Figure 3, all HERV-K dUTPase recombinant proteins, except the human dUTPase, induced NF-κB activity, ranging from 4.5- to 31.5-fold. Interestingly, the recombinant HERV-K dUTPase mutant protein Δ142–171, which lacks the C-terminal 30 amino acids (AAs), caused the highest level of NF-κB activation (31.5-fold), whereas deleting the C-terminal 22 AAs impaired the ability of the HERV-K dUTPase mutant protein Δ155–171 to activate NF-κB relative

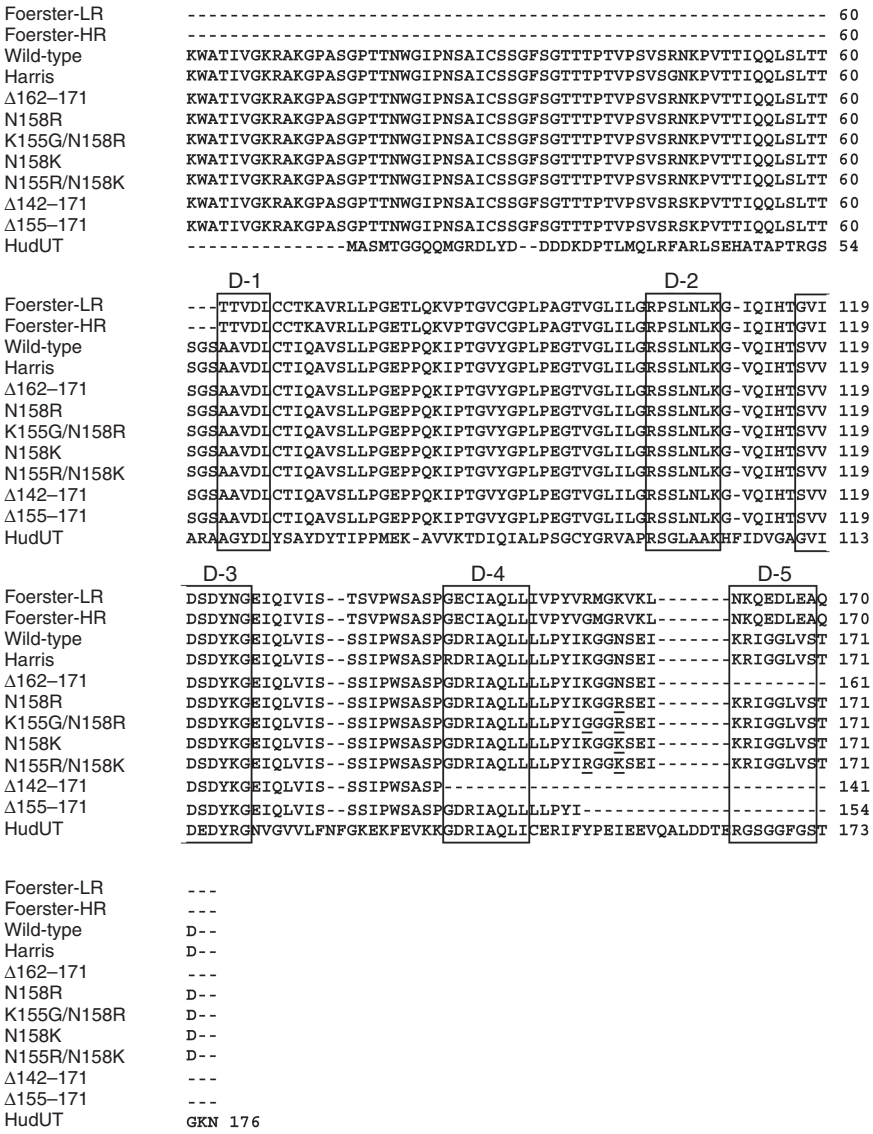

Figure 1. Deoxyuridine triphosphate nucleotidohydrolase (dUTPase) protein blast alignment. Human endogenous retrovirus K (HERV-K) consensus, as described by Harris *et al.* (1997), low-risk haplotype and high-risk haplotype, as described by Foerster *et al.* (2005), HERV-K dUTPase recombinant proteins reflecting wild-type, low-risk (N158K and K155R/N158K) and high-risk (N158R and K155G/N158R) haplotypes, as well as various deletion mutations (Δ162–171; Δ155–171, Δ142–171) used in this study, and the human dUTPase (HudUT). Boxes D-1–D-5 represent the conserved domains of the dUTPase protein family.

to that induced by wild-type HERV-K dUTPase protein. No activation of NF-κB by the wild-type HERV-K dUTPase protein was observed in HEK293 cells lacking TLR2, which suggests that NF-κB activation is TLR2 dependent. These data demonstrate that HERV-K dUTPase proteins activate NF-κB through TLR2 and that the C-terminal end of the HERV-K dUTPase protein is important in HERV-K dUTPase-mediated activation of TLR2 and NF-κB.

HERV-K dUTPase induces cytokine/chemokine secretion in human primary cells

Recent studies have implicated dendritic cells as being the central cell mediator in psoriasis as they activate T cells and produce cytokines/chemokines that amplify the immune response (Nestle *et al.*, 2009; Zaba *et al.*, 2009; Elder *et al.*, 2010). To determine whether HERV-K dUTPase proteins could induce the secretion of cytokines/chemokines in cells known to be involved in psoriasis (Lowes *et al.*, 2007; Nickoloff *et al.*, 2007; Hedrick *et al.*, 2009), proteome array studies were conducted in normal hDCs and keratinocytes. As shown in Figure 4a–f, treatment of primary hDCs with wild-type and mutant HERV-K dUTPase recombinant proteins resulted in the increased production of cytokines tumor necrosis factor-α, IL-23, IL-17, IL-8, IL12p40, and INF-γ. In addition, HERV-K dUTPase proteins also induced the production of chemokines CCL20 and RANTES. As shown in Figure 5a–c, there was a modest, but in some cases a statistically significant, increase in the production of IL-8,

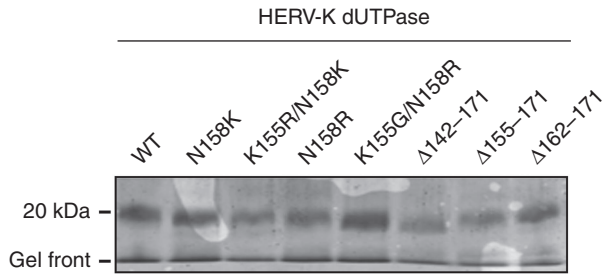

Figure 2. SDS-PAGE of purified human endogenous retrovirus K (HERV-K) deoxyuridine triphosphate nucleotidohydrolase (dUTPase). SDS-PAGE was performed as described in Materials and Methods using a 10% gel. Approximately 25 μg of purified protein was added to each lane. The following electrophoresis gels were stained with Lab Safe Gel Blue according to the manufacturer's instructions. Lane 1: wild-type (WT) HERV-K dUTPase; lane 2: N158K; lane 3: K155R/N158K; lane 4: N158R; lane 5: K155G/N158R; lane 6: Δ142–171; lane 7: Δ155–171; lane 8: Δ162–171.

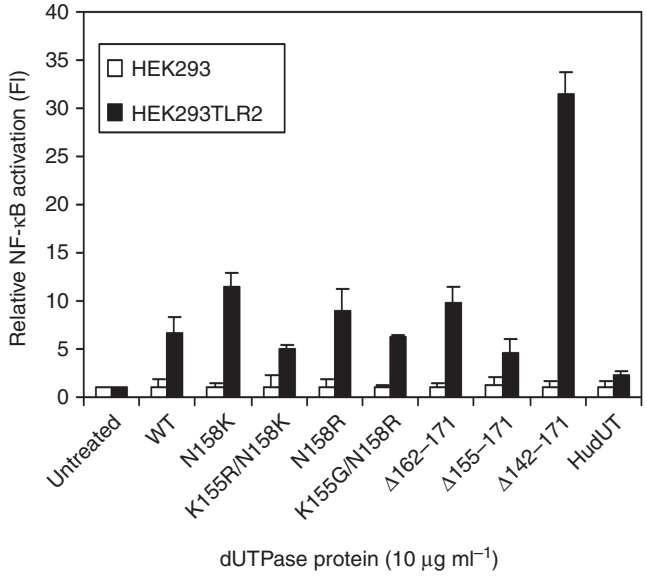

Figure 3. Human endogenous retrovirus K (HERV-K) deoxyuridine triphosphate nucleotidohydrolase (dUTPase) modulates innate immunity. HERV-K dUTPase proteins activate NF-κB through TLR2 in HEK293TLR2-expressing cells, as determined by luciferase assay. HEK293TLR2 and wild-type (WT) HEK293 cells were transiently transfected with NF-κB luciferase reporter plasmid, as described previously (Ariza *et al.*, 2009). After 24–36 hours, cells were treated with various HERV-K dUTPase proteins (10 μg ml^{-1}), human dUTPase control protein (10 μg ml^{-1}), and zymosan (10 μg ml^{-1}, data not shown), or left untreated for 8 hours, and luciferase reporter gene activity was measured. Data are expressed as the mean fold induction ± SD relative to control levels. Values represent the average of three independent experiments. HEK293, human embryonic kidney 293; TLR2, Toll-like receptor 2.

CCL20, transforming growth factor-α, RANTES, IL-12p40, IL-1β, IL-17, and IL-23 in the supernatant of HERV-K dUTPase-treated keratinocytes.

DISCUSSION

Foerster *et al.* (2005) identified two single-nucleotide polymorphisms in the *HERV-K dUTPase* gene located at the PSORS1 locus that distinguished low- and high-risk haplotypes associated with psoriasis and suggested that the retroviral dUTPase was a candidate gene for the PSORS1 mutation. As a functional role for HERV-K dUTPase in the pathophysiology of psoriasis has never been established, we decided to explore whether HERV-K dUTPase possessed immunomodulatory properties similar to those reported for the Epstein–Barr virus-encoded dUTPase (Glaser *et al.*, 2006; Waldman *et al.*, 2008; Ariza *et al.*, 2009).

In this study, we demonstrate that HERV-K dUTPase can modulate innate and adaptive immune responses in hDCs and keratinocytes through the activation of TLR2, NF-κB, and the secretion of proinflammatory T_H1/T_H17 cytokines, which, to our knowledge, has not been reported yet. Homotrimeric and monomeric dUTPases contain five hallmark conserved sequence domains that are essential for the formation of the catalytic sites (McGeoch, 1990; Larsson *et al.*, 1996; Mol *et al.*, 1996; Dauter *et al.*, 1999; Harris *et al.*, 1999; Tarbouriech *et al.*, 2005). As there is considerable sequence variation in conserved domain 5 of the β-retrovirus dUTPases, of which HERV-K is a member, when compared with homotrimeric and monomeric dUTPases from other species (Harris *et al.*, 1997; Fiser and Vertessy, 2000; Foerster *et al.*, 2005), we explored the possibility that the C-terminal region of the HERV-K dUTPase might be important for the immunomodulatory properties of the protein. The wild-type and mutant recombinant HERV-K dUTPases used in this study lacked dUTPase activity, which confirms the original studies by Harris *et al.* (1997) and is consistent with structural and functional data indicating that the highly conserved phenylalanine residue in conserved domain 5, which is absent in the HERV-K dUTPases used in this study, is required for catalytic activity (Mol *et al.*, 1996; Dauter *et al.*, 1999; Harris *et al.*, 1999; Tarbouriech *et al.*, 2005; Freeman *et al.*, 2009; Takacs *et al.*, 2009). Various studies of the C-terminal end of monomeric and homotrimeric dUTPases, containing conserved domain 5, have demonstrated that this region is highly flexible because of its role in enzyme catalysis (Nord *et al.*, 2001; Freeman *et al.*, 2009; Takacs *et al.*, 2009). The HERV-K dUTPase mutant protein Δ142–171, lacking the conserved domains 4 and 5 and the hinge proline 152 (Takacs *et al.*, 2009), caused the highest NF-κB activation level, which correlated with the greatest induction of the cytokines/chemokines tested. Conversely, deletion of the terminal 17 AAs (Δ155–171) or domain 5 (Δ162–171) had little, if any, effect on altering the ability of the protein to activate NF-κB, relative to the wild type. These results suggest that either a domain is located between AAs 142 and 154 that inhibits the interaction of HERV-K dUTPase with TLR2 or, conversely, that deletion of the terminal 30 AAs, containing conserved domains 4 and 5 and the hinge proline 152, removes the C-terminal flexible arm of the protein, which could sterically interfere with HERV-K dUTPase interacting with TLR2. Thus, although additional studies are required to distinguish these possibilities, our data demonstrate that the immunomodulatory activity of HERV-K dUTPase is a distinct function independent from its catalytic activity and suggest that the C-terminal 30 AAs have a critical

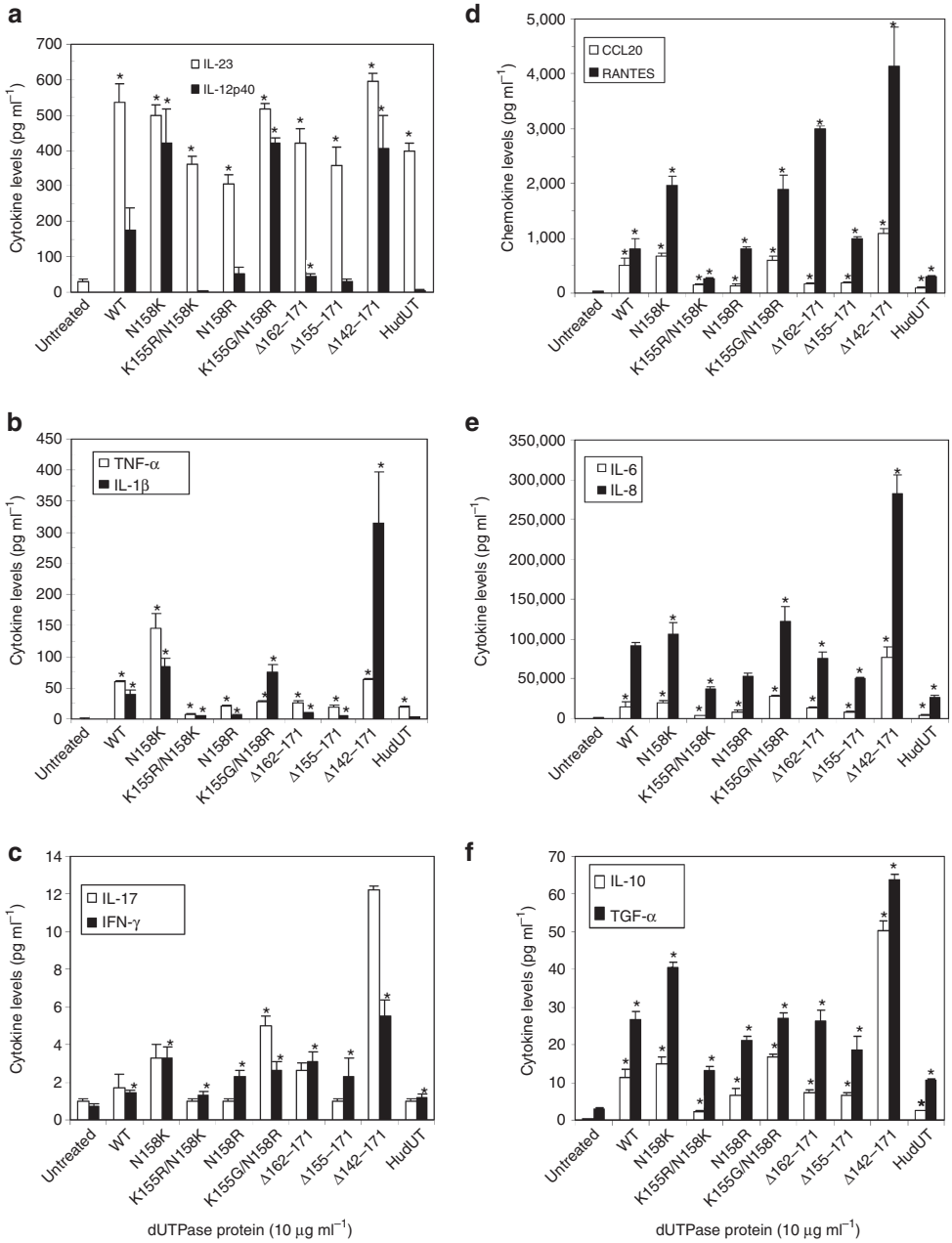

Figure 4. Human endogenous retrovirus K (HERV-K) deoxyuridine triphosphate nucleotidohydrolase (dUTPase) proteins stimulate the secretion of T_H1, T_H17 cytokines in human dendritic/Langerhans-like cells (hDCs). HERV-K dUTPase proteins differentially induce the secretion of T_H1, T_H17 cytokines in hDCs following a 24-hour treatment, as determined by proteome array. (**a**) IL-23 and IL12p40; (**b**) tumor necrosis factor (TNF)-α and IL-1β; (**c**) IL-17 and IFN-γ; (**d**) CCL20 and RANTES; (**e**) IL-8 and IL-6; (**f**) IL-10 and transforming growth factor (TGF)-α. Cytokine/chemokine concentrations represent the average ± SD (pg ml^{-1}) from two independent experiments ($n = 4$). *$P < 0.05$.

role in regulating the immunomodulatory properties of HERV-K dUTPase.

Blast analysis using the wild-type *HERV-K dUTPase* gene demonstrated that there are 14 HERV-K dUTPase sequences located in the human genome (including the one located at the PSORS1 locus) that exhibit a high level of homology (89–99%) to our wild-type DNA sequence. As the wild-type *HERV-K dUTPase* gene used in our studies was generated from human genomic DNA using gene-specific primers, we cannot state that the amplified *HERV-K dUTPase* gene corresponds to the one in the PSORS1 region. Somewhat interestingly, however, is that these HERV-K dUTPase genes map to regions previously identified as containing psoriasis susceptibility genes, as well as other loci identified in various autoimmune diseases (Johanneson *et al.*, 2002; Bowcock, 2005; Barton *et al.*, 2008; Chang *et al.*, 2008; Hollox *et al.*, 2008; De Jager *et al.*, 2009; Gateva *et al.*, 2009; Nair *et al.*, 2009; Zhang *et al.*, 2009; Huffmeier *et al.*, 2010; Stuart *et al.*, 2010; Sun *et al.*, 2010; Franke *et al.*, 2010; Roberson and Bowcock, 2010).

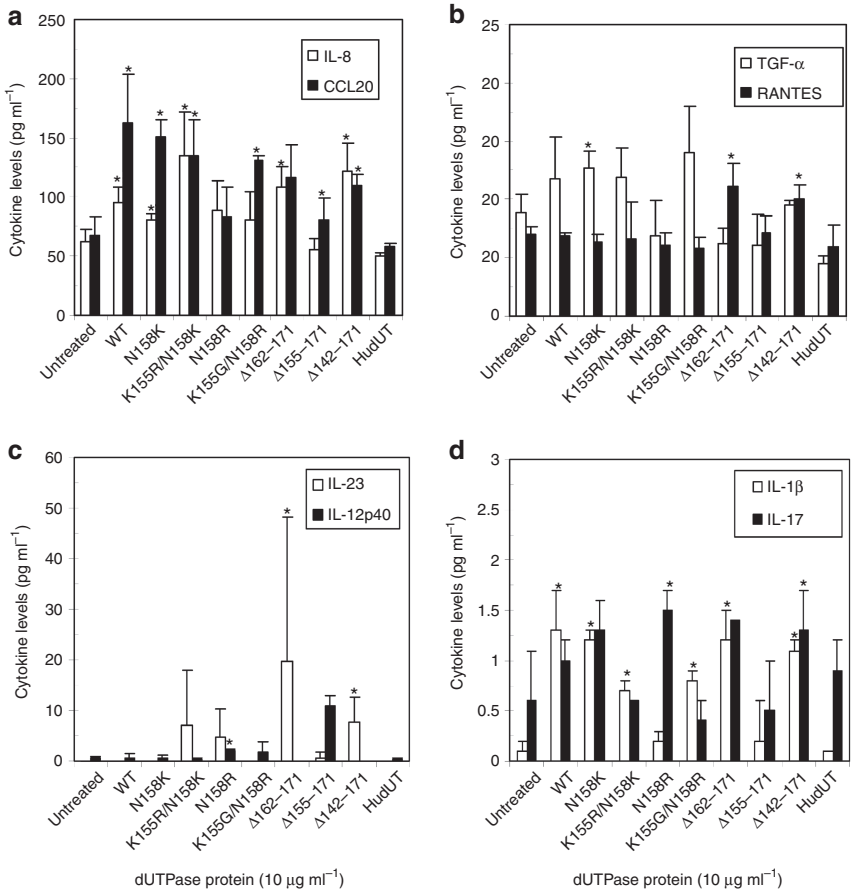

Figure 5. Effect of human endogenous retrovirus K (HERV-K) deoxyuridine triphosphate nucleotidohydrolase (dUTPase) proteins on T_H1, T_H17 cytokine secretion in human primary keratinocytes. HERV-K dUTPase proteins differentially induce the secretion of chemokines and proinflammatory cytokines in primary keratinocytes following a 24-hour treatment, as determined by proteome array. (**a**) IL-8 and CCL20; (**b**) transforming growth factor (TGF)-α and RANTES; (**c**) IL-23 and IL-12p40; (**d**) IL-1β and IL-17. Cytokine/chemokine concentrations represent the average ± SD (pg ml^{-1}) from two independent experiments ($n = 4$). *$P < 0.05$.

As we did not use non-lesional or lesional skin from patients with psoriasis in these studies, it is not possible to establish a role for HERV-K dUTPase in psoriasis pathophysiology. However, several studies have reported the transcriptional activation of HERV sequences in keratinocytes (Hohenadletal *et al.*, 1999) and in skin from patients with psoriasis (Bessis *et al.*, 2004; Moles *et al.*, 2005). Furthermore, Foerster *et al.* (2005) demonstrated that the *HERV-K dUTPase* gene located at the PSORS1 locus is expressed in non-lesional skin and in peripheral blood mononuclear cells from patients with psoriasis, as well as in skin from healthy controls. Similarly, whether there are sufficient amounts of HERV-K dUTPase secreted to initiate the TLR2 cascade described in this study remains unknown. Although HERV-K dUTPase was described as having an intracellular location (Harris *et al.*, 2000), there have not been any additional studies to determine whether this protein is secreted from cells. However, there is an accumulating body of evidence demonstrating that virus-encoded macromolecules are secreted in exosomes (Flanagan *et al.*, 2003; Gould *et al.*, 2003; Pelchen-Matthews *et al.*, 2004; Fang *et al.*, 2007; Balaj *et al.*, 2011; Izquierdo-Useros *et al.*, 2011; Vallhov *et al.*, 2011), and defective particles from HERVs have been found in microvesicle populations (Voisset *et al.*, 2008) and in the plasma and urine of people with various autoimmune diseases (Büscher *et al.*, 2005; Dewannieux *et al.*, 2006; Contreras-Galindo *et al.*, 2008; Blank *et al.*, 2009; Kakalacheva *et al.*, 2011), including psoriasis (Iversen, 1990). However, additional studies are required to address this possibility.

The results presented in this study demonstrate that the stimulation of TLR2 by HERV-K dUTPase results in the activation of NF-κB signaling pathways and in the secretion of proinflammatory T_H1/T_H17 cytokines in both hDCs and keratinocytes. Interestingly, HERV-K dUTPase-mediated activation of NF-κB and induction of a T_H1/T_H17 cytokine response resemble what is reported to occur in psoriatic skin (Nair *et al.*, 2009; Zaba *et al.*, 2009). Although additional studies are needed to determine the expression levels of the *PSORS1 HERV-K dUTPase* gene, to demonstrate a relationship between protein levels in the skin with disease progression and to correlate mutations in this gene with disease severity, the results of this study provide evidence supporting an endogenous retrovirus protein, the

HERV-K-encoded dUTPase, as a potential contributing factor in the pathophysiology of psoriasis.

MATERIALS AND METHODS

Amplification of the consensus sequence

PCR amplification of the *HERV-K dUTPase* gene was performed using the forward and reverse primer set (125 pmol of each), as described by Harris *et al.* (1997), human genomic DNA (140 ng; Clontech, Mountain View, CA), high-fidelity PCR supermix (Invitrogen, Carlsbad, CA), and the following PCR conditions: denaturation at 94 °C for 3 minutes (1 cycle), followed by 35 cycles of 94 °C for 30 seconds, 50 °C for 30 seconds, 72 °C for 1 minute, and 1 cycle at 72 °C for 10 minutes. Using these PCR conditions, a single DNA fragment of approximately 521 bp was amplified. The PCR product was purified using the QIAquick gel extraction kit (QIAGEN), cloned into the pTrcHis Topo TA expression vector, and the sequence verified by DNA sequencing analysis at Sequetech Corporation (Mountain View, CA).

Mutant construction

HERV-K dUTPases containing point mutations reflecting the high- and low-risk haplotypes of psoriasis were generated by site-directed mutagenesis using the QuikChange Lightning Mutagenesis system (Stratagene, Santa Clara, CA) and the specific primer sets for the high-risk (N159R: 5′-ATTATTACTCCTGCCTTATATTAAGGTTGGA AGGAGTGAGATAAAAAGAACAAGAG-3′; N159R-antisense: 5′-CT CTTGTTCTTTTTATCTCACTCCTTCCAACCTTAATATAAGGCAGGAG TAATAAT-3′) and low-risk (N159K: 5′-TTACTCCTGCCTTATATTAAG GTTGGAAAGAGTGAGATAAAAAGAAC-3′; N159K-antisense: 5′-GT TCTTTTTATCTCACTCTTTCCAACCTTAATATAAGGCAGGAGTAA-3′) haplotypes, following the recommendations of the manufacturer. The following PCR conditions were used: one cycle at 95 °C for 2 minutes, followed by 18 cycles of 95 °C for 20 seconds, 60 °C for 10 seconds, and 68 °C for 2.5 minutes, and one cycle at 68 °C for 5 minutes. Amplified products were *Dpn*I digested and screened for the β-galactosidase (β-gal +) phenotype. DNA was then purified and the AA change from asparagine to either arginine (high risk) or lysine (low risk) was verified by sequence analysis. dUTPases containing the point mutations reflecting the high- and low-risk haplotypes have been designated N158R and N158K, respectively. Dual-point mutation constructs of HERV-K dUTPase (N155R/N158K and N155R/N158R) were also generated using the forward primer HERV-K-FL: 5′-GGGCTTGTAAGCACTGATTGAGAATTCCTGTCA-3′ and the following reverse primer sets: for the high-risk HRDM: 5′-CCTTATATTGGGGTGGAAGGAGTGAGATATAGGAATTCAGCT GC-3′; for the low-risk LRDM: 5′-CCTTATATTAGGGGTGGAAAG AGTGAGATATAGGAATTCAGCTGC-3′.

HERV-K dUTPase deletion mutations lacking the C-terminal 10 AAs (Δ162–171), the C-terminal 17 AAs (Δ155–171), and the C-terminal 30 AAs (Δ142–171) were generated by PCR using HERV-K-FL forward and the reverse HervdutΔ161: 5′-GGGGAAATA GTGAGATATAGGAATTCAGCTGC-3′; HervdutΔ154: 5′-GCTCAAT TATTACTCCTGCCTTATATTTAGGAATTCACCGG-3′; and HervdutΔ141: 5′-ATTCCTTGGAGTGCCAGTCCATGAGAATTCATTG
CT primer sets, respectively.

Purification of proteins

The recombinant HERV-K-encoded dUTPases were purified under native conditions using HisPur cobalt spin columns (3 m1; Pierce, Rockford, IL). Briefly, *E. coli* BL21 containing the various expression constructs was grown in Luria Bertani medium and induced with 1 M isopropyl β-D-1 thiogalactopyranoside, as we have described (Glaser *et al.*, 2006). Bacteria pellets were collected by low-speed centrifugation, resuspended in equilibration buffer (0.05 M sodium phosphate, 0.3 M NaCl, and 0.01 M imidazole, pH7.4), and lysed by ultrasonication. The resulting homogenate was centrifuged (15,000 × *g*, 30 minutes at 4 °C), and the supernatant was applied to a HisPur cobalt spin column. The recombinant proteins were eluted as described by the manufacturer. Fractions containing the recombinant protein were combined and concentrated using Centriprep-10 (Amicon, Beverly, MA). SDS-PAGE and purity of the protein preparations were determined as we have described (Studebaker *et al.*, 2005; Glaser *et al.*, 2006). Several assays were performed to eliminate the possibility that our purified dUTPase preparations were contaminated with bacterial components. High-sensitivity Quant-iT kits (Molecular Probes, Carlsbad, CA) were used to detect possible DNA and RNA contamination. The Quant-iT DNA assay can detect double-stranded DNA down to 1 ng ml^{-1}, whereas the Quant-iT RNA assay has a detection limit of 5 ng ml^{-1}. For the detection of peptidoglycan, the silkworm larvae plasma assay (Wako Chemicals, Richmond, VA) was used. The silkworm larvae plasma assay has a detection limit of 10 pg ml^{-1} of peptidoglycan. To determine lipopolysaccharide contamination in our protein preparations, dUTPase preparations were assayed for lipopolysaccharide using the Limulus Amebocyte Lysate Kit (BioWhittaker, Walkersville, MD), which can detect endotoxin concentrations of 0.06 IU ml^{-1}. The lipopolysaccharide level in these preparations ranged from 2 to 52 IU ml^{-1}, with the highest concentration occurring in the human dUTPase preparation. The purified dUTPase preparations were stored at −20 °C at stock concentrations of 0.4–0.5 mg ml^{-1}. Protein concentration was determined with the Coomassie Brilliant Blue dye-binding assay (Bio-Rad Laboratories, Hercules, CA), using BSA as the standard. To determine whether the HERV-K-encoded dUTPase proteins possessed functional enzymatic activity, fractions were assayed using a standard DE81 filter disc assay. The detection limit of this assay, which has been described previously (Williams, 1985; Glaser *et al.*, 2006), is 0.001 unit of dUTPase activity under standard assay conditions for 1 hour at 37 °C.

Cell culture

HEK293 cells stably expressing human TLR2 (Invivogen, San Diego, CA) were maintained in DMEM-supplemented medium, as described (Ariza *et al.*, 2009). Human primary hDCs were purchased from MatTek Corporation (Ashland, MA). hDCs were generated from human umbilical cord blood CD34 + progenitor cells and cultured using specially formulated medium, DC-100-MM (MatTek Corporation), containing a cytokine cocktail designed to induce differentiation of the CD34 + progenitor cells into dendritic cells. These cells express key markers including CD1a, HLA-DR, co-stimulatory molecules, Birbeck granules, and surface markers characteristic of both plasmacytoid and myeloid dendritic cells (Ayehunie *et al.*, 2003). Normal human epidermal adult keratinocytes (cat no. CC-2501A) were obtained from Lonza Biologics (Hopkinton, MA) and maintained in KGM-2 medium.

Luciferase reporter gene assays

HEK293 cells (2.5×10^5) were seeded into 12-well plates and 24 hours later transiently transfected with 0.5 μg of pNFκB-Luc (a gift

from Dr Douglas Golenbock, the University of Massachusetts Medical School), 8 ng of pRL-TK reporter vectors (Promega, Madison, WI), or with empty vector as described (Ariza *et al.*, 2009). At 24–36 hours after transfection, cells were treated with various concentrations of purified HERV-K dUTPase (10 μg ml^{-1}) for 8 hours or left untreated. Zymosan (10 μg ml^{-1}), a TLR2 ligand, was used as a positive control. After treatment, cell lysates were prepared and reporter gene activities were measured using the dual-luciferase reporter assay system (Promega). Data were normalized for transfection efficiency by measuring Renilla luciferase activity and expressed as mean relative stimulation ± SD.

Cytokine/chemokine proteome array

Primary hDCs and keratinocytes (3×10^5) were treated with consensus HERV-K dUTPase or point/deletion mutation protein variants (10 μg ml^{-1}) for 24 hours and the levels of 12 cytokines/chemokines in cell supernatants of treated and control samples were measured using SearchLight proteome arrays (Aushon BioSystems, Billerica, MA). Briefly, samples were incubated for 1 hour on the array plates that were pre-spotted with capture antibodies specific for each protein biomarker. The bound proteins were detected using a biotinylated detection antibody, followed by the addition of streptavidin-horseradish peroxidase, and finally a chemiluminescent substrate. The plates were immediately imaged using the SearchLight imaging system, and data were analyzed using SearchLight Array Analyst software. The amount of luminescent signal produced was proportional to the amount of each protein present in the original standard or sample. Concentrations are extrapolated off a standard curve and represent the average ± SD of an *n* of 4.

Statistical analysis

Statistical analyses were performed using a paired two-sample *t*-test for the means. Results are shown as mean ± SEM. Two-tailed *P*-values have been reported when significant.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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