



Human endogenous retrovirus W family envelope protein (HERV-W env) facilitates the production of TNF- α and IL-10 by inhibiting MyD88s in glial cells

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Abstract

Human endogenous retrovirus W family envelope protein (HERV-W env) is associated with several neurological and psychiatric disorders, including multiple sclerosis (MS) and schizophrenia. Clinical studies have demonstrated a common link between inflammatory abnormalities and HERV-W env in neuropsychiatric diseases. Nonetheless, the molecular mechanisms by which HERV-W env mediates neuroinflammation are still unclear. In this study, we found that HERV-W env significantly increased the mRNA and protein levels of TNF- α and IL-10 in U251 and A172 cells. HERV-W env also induced a notable increase in Toll-like receptor 4 (TLR4). Knockdown of TLR4 impaired the expressions of TNF- α and IL-10 induced by HERV-W env. Overexpression of HERV-W env led to the upregulation of MyD88 but caused a decrease in MyD88s. MyD88s overexpression suppressed the expressions of TNF- α and IL-10 induced by HERV-W env. These findings indicate that HERV-W env upregulates the expressions of IL-10 and TNF- α by inhibiting the production of MyD88s in glial cells. This work sheds light on the immune pathogenesis of HERV-W env in neuropsychiatric disorders.

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Xiuling Wang, Xiulin Wu and Jin Huang contributed equally to this work.

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Introduction

Human endogenous retroviruses (HERVs) account for 8% of the human genome, and 3173 HERV sequences have been identified. Of these, 1214 are segregated into 39 canonical clades (groups), belonging to class I (gamma- and epsilon-like), class II (beta-like), and class III (spuma-like). The other 1959 noncanonical HERVs include 31 less distinct groups [1, 2]. Most HERVs are defective, containing major deletions or nonsense mutations. Only a few of HERVs have intact open reading frames (ORFs). These HERVs encode functional proteins, some of which play pivotal roles in placentation and gene regulation [3, 4]. HERV-W, one of the HERV groups, was initially discovered in multiple sclerosis (MS) patients [5]. HERV-W env, located on chromosome 7, encodes the HERV-W envelope protein syncytin-1. Previous investigations have shown that various environmental factors, including viruses and caffeine, regulate HERV-W env mRNA and protein expression [6, 7]. Clinical studies have revealed an increase in the amount of HERV-W env in the serums of schizophrenia and multiple sclerosis (MS) patients [8, 9]. Moreover, recent research findings suggest that HERV-W env has potential immunomodulatory properties in the nervous system [10, 11] and participates in the development of several neurological and psychiatric disorders [12, 13]. Expression of pro-inflammatory

cytokines such as interleukin (IL)-1 β and IL-6 can be induced by HERV-W env in peripheral blood mononuclear cells (PBMCs) and microglial cells [14, 15]. Nevertheless, the role of HERV-W env in neuropsychiatric diseases is still unclear.

Inflammation is a biological response to harmful stimuli [16, 17]. Chronic inflammation contributes to various diseases, such as atherosclerosis [18], rheumatoid arthritis [19], cancer [20, 21], and even schizophrenia [22]. Neuroinflammation is chronic inflammation, together with abnormal expression of inflammation-related cytokines in the nervous system [22–24]. The IL-10 gene is a candidate gene for susceptibility to schizophrenia, and IL-10 is a cytokine with pleiotropic effects in inflammation [25, 26]. Tumour necrosis factor- α (TNF- α) is one of the inflammatory markers that is elevated in patients with schizophrenia [27, 28]. Nevertheless, it is still unknown whether HERV-W env is involved in the regulation of IL-10 and TNF- α .

TLR4 (Toll-like receptor 4), a pattern-recognition receptor (PRR), plays a vital role in the innate immune system. TLR4 is a pivotal player in the inflammatory response, initiating an intracellular signal cascade via the NF- κ B-dependent pathway [29]. A recent study showed that HERV-W env regulates the inflammatory effect via TLR4 [30]. Accumulating evidence indicates that HERV-W env induces TLR4 activation [31]. Meanwhile, neutralizing antibodies against TLR4 were shown to impair HERV-W-env-induced inflammation in human monocytes [15, 22]. Myeloid differentiation primary response 88 (MyD88) is a universal adapter protein for TLR4. It triggers the activation of the NF- κ B pathway and the subsequent production of pro-inflammatory cytokines [32]. MyD88s, a splice variant of MyD88, lacks the small intermediate domain (ID) separating the N-terminal death domain (DD) and the C-terminal Toll/interleukin-1 receptor (TIR) domain [33]. MyD88s blocks TLR4-mediated NF- κ B activation and negatively regulates the inflammatory response [34].

In this study, we demonstrated that HERV-W env induced the production of the inflammation-related cytokines IL-10 and TNF- α in glial cells. Moreover, we found that MyD88s was capable of inhibiting the production of inflammation-related cytokines induced by HERV-W env. These findings suggested a potential regulatory role of HERV-W env in neuroinflammation. We also discovered a MyD88s-dependent mechanism by which inflammation is regulated by HERV-W env.

Materials and methods

Plasmid construction

The HERV-W env plasmid was constructed as described previously [35]. The pCMV-Myc-MyD88s plasmid was

constructed as follows: The upstream fragment of the MyD88 gene (nt 110–557) was amplified using the primers 5'-GGG AAT TCT CTC GGA AAG CGA AAG CC-3' and 5'-CCA AGC TTA TGC TGG GTC CCA GCT CC-3'. The downstream fragment (nt 692–1243) was amplified using the primers 5'-CCA AGC TTG CGT TTC GAT GCC TTC AT-3' and 5'-CGG GTA CCA GAG CAC AGA TTC CTC CTA C-3'. The two fragments were then linked and cloned into the mammalian expression vector pCMV-Myc, which contains an N-terminal c-Myc epitope tag and a CMV promoter. Specific siRNAs against human TLR4 (siTLR4) and a negative control siRNA (siNC) were purchased from the Guangzhou RiboBio Co., Ltd. (Guangzhou, China). The target sequence of siTLR4 was GTGCAATTTGACCATTGAA.

Cell culture and transfection

Two human glioma cell lines, U251 and A172, were purchased from the American Type Culture Collection (ATCC) and maintained in DMEM (GIBCO, California, USA) supplemented with 10% fetal bovine serum (FBS) (GIBCO, California, USA) and 100 U of penicillin/streptomycin per ml at 37°C with 5% CO₂.

Transient transfection was carried out using Lipofectamine® LTX and PLUS™ (Invitrogen, Carlsbad, CA, USA) following the manufacturer's protocol. Briefly, 1 μ g of plasmid DNA was mixed with 1 μ l of PLUS™ Reagent in Opti-MEM medium in a 12-well format, and subsequently incubated with 3 μ l of Lipofectamine LTX reagent for 30 min. Next, the DNA-lipid complex was added dropwise to the cells. Cells were harvested 48 hours after transfection for further study.

For cotransfection, 0.5 μ g of pCMV-env plasmid and 0.5 μ g of pCMV-Myc-MyD88s plasmid were used with Lipofectamine® LTX and PLUS™ Reagent to transfect cells in a 12-well format. Cells were harvested 48 hours after transfection for further study. Untransfected cells and the cells that were cotransfected with plasmids pCMV-env and pEGFP were used as controls.

Cells were also cotransfected with the plasmid pCMV-env and siTLR4. Briefly, 0.5 μ g of pCMV-env plasmid and 10 nM specific siTLR4 were co-transfected using Lipofectamine® LTX and PLUS™ Reagent in a 12-well format. Cells were harvested 48 hours after transfection for further study. Untransfected cells and the cells that were cotransfected with plasmid pCMV-env and siNC were used as controls.

Analysis of mRNA expression

Total RNA was isolated from cultured cells using the TRIzol Reagent (Invitrogen, Carlsbad, CA, USA) and treated with DNase I (Fermentas, Massachusetts, USA) to remove

genomic DNA. Reverse transcription was carried out using the MMLV reverse transcriptase kit (Promega, Madison, WI, USA) according to the manufacturer's protocol. Quantitative real-time PCR (Q-PCR) was performed with SYBR[®] Select Master Mix (Invitrogen, Carlsbad, CA, USA) using an iCycler System (Bio-Rad, Hercules, CA, USA). The $2^{-\Delta\Delta CT}$ method was used to analyze the relative difference in gene expression. β -actin was used as an internal control to normalize the expression of mRNA levels between different samples. The sequences of this primer that were used are shown in Table S1.

ELISA assay

The culture supernatants were collected from the treated cells. The intracellular contents were obtained by lysing the cells with Cell Lysis Buffer P0013 (Beyotime Institute of Biotechnology, Shanghai, China). The levels of IL-10 and TNF- α in culture supernatants and cell lysates were determined using an OptEIA ELISA kit (Becton, Dickinson and Company, New Jersey, USA) according to the manufacturer's instructions.

Western blot

Western blot analysis was performed as described previously [36] with slight modifications. Equal amounts of protein were separated by SDS-PAGE and transferred to nitrocellulose filter (NC) membranes (Millipore, Billerica, MA). The membranes were then blocked with 5% skim milk and incubated with specific anti-TNF- α (ab183218) or anti-IL-10 (ab52909) antibodies (Abcam, Cambridge, MA) at a dilution of 1:1000. The expression levels of the targeted proteins were quantitated relative to β -actin in the same sample and normalized to the respective control group, for which the value of the band density was arbitrarily set to 1.

Statistical analysis

All results are shown as the mean \pm SD (standard deviation) from at least three independent experiments. One-way analysis of variance and Student's *t*-test were used to analyze the data. The mRNA and protein levels were calculated as the fold change relative to the control group. A *p*-value less than 0.05 was considered to represent a significant difference.

Results

HERV-W env increases the TNF- α /IL-10 ratio in U251 and A172 cells

We first investigated whether HERV-W env could regulate the expressions of the inflammation-related cytokines TNF- α and IL-10 in glial cells. We used the human glioma cell lines U251 and A172, which are the most widely used in neuroinflammation research [37, 38]. We transfected the U251 and A172 cells with the plasmid encoding HERV-W env, and the transfection efficiency proved to be robust (Fig. S1). Using quantitative PCR, we found that TNF- α mRNA levels were elevated in HERV-W-overexpressing U251 and A172 cells, by 1.7- and 5.7-fold, respectively (Fig. 1A and D). Meanwhile, western blot showed that the overexpression of HERV-W env increased the protein levels of TNF- α in U251 and A172 cells (Fig. 1B, C, E, and F). ELISA assay confirmed that the level of TNF- α protein in the culture supernatant of U251 cells overexpressing HERV-W env was significantly higher than in the control (17.6 pg/mL and 4.0 pg/mL, respectively) (Fig. 1G, *p* < 0.01). HERV-W env overexpression also led to a high protein concentration of 12.5 pg/mL of TNF- α in the culture supernatant of A172 cells, compared to 3.9 pg/mL in the control (Fig. 1I, *p* < 0.01). Similarly, HERV-W env also increased the amount of intracellular TNF- α in U251 and A172 cells (Fig. 1H and J).

The mRNA levels of IL-10 also increased by 1.8- and 1.7-fold, respectively, in U251 and A172 cells transfected with HERV-W env (Fig. 2A and D). Consistently, the protein levels of IL-10 also increased after overexpression of HERV-W env in glial cells (Fig. 2B, C, E, and F). The western blot results were corroborated by ELISA, showing that HERV-W env promoted IL-10 expression, with protein concentrations of 335.8 and 274.7 pg/mL in the culture supernatant of U251 and A172 cells, respectively, and 224.7 and 158.1 pg/mL in that of pCMV-transfected U251 and A172 cells, respectively (Fig. 2G and I, *p* < 0.05). Similar increases in IL-10 in the intracellular fluid were observed in U251 and A172 cells overexpressing HERV-W env (Fig. 2H and J). A time-course analysis showed that HERV-W env overexpression elevated the protein levels of TNF- α and IL-10 at different time points in U251 and A172 cells, which peaked at 36 hours after transfection (Fig. S2). These findings suggested that HERV-W env upregulated the expressions of the inflammation-related cytokines TNF- α and IL-10 in glial cells.

Because the TNF- α /IL-10 ratio reflects the balance of pro- and anti-inflammatory cytokines [39, 40], we measured the TNF- α /IL-10 ratio in the supernatants of glial cells after overexpression of HERV-W env. The results

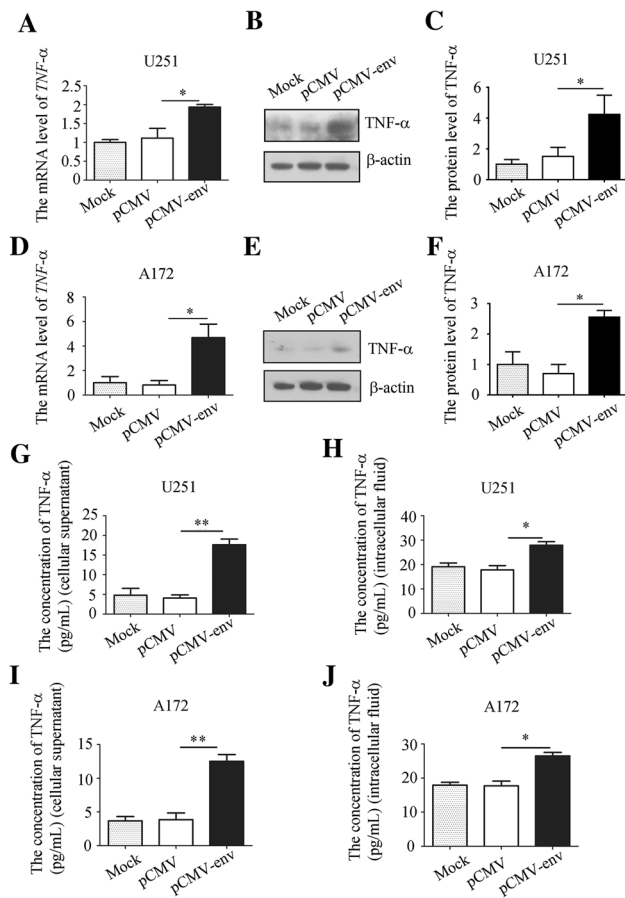


Fig. 1 HERV-W env increases the expression of TNF- α in U251 and A172 cells. (A and D) mRNA expression levels of TNF- α in HERV-W-env-overexpressing U251 and A172 cells. (B and C) A representative western blot and the protein expression level of TNF- α in HERV-W-env-overexpressing U251 cells. (E and F) A representative western blot and the protein expression level of TNF- α after transfection of A172 cells with HERV-W env. (G and H) Expression levels of TNF- α in the culture supernatant and intracellular fluid of U251 cells, measured by ELISA. (I and J) Expression levels of TNF- α in the culture supernatant and intracellular fluid of A172 cells, measured by ELISA. The data represent the mean \pm standard deviation of three independent experiments. *, $P < 0.05$; **, $P < 0.01$ compared to pCMV control

indicated that overexpression of HERV-W env significantly increased the TNF- α /IL-10 ratio when compared to the control (Fig. 2K and L), suggesting that HERV-W env might trigger a pro-inflammatory response.

HERV-W env increases the expression of TLR4 in U251 and A172 cells

As the TLR4/MyD88 pathway predominantly mediates the production of inflammation-related cytokines, including IL-10 and TNF- α [41, 42], we explored whether the TLR4/MyD88 signal pathway contributed to the release of increased TNF- α and IL-10 induced by HERV-W env. The

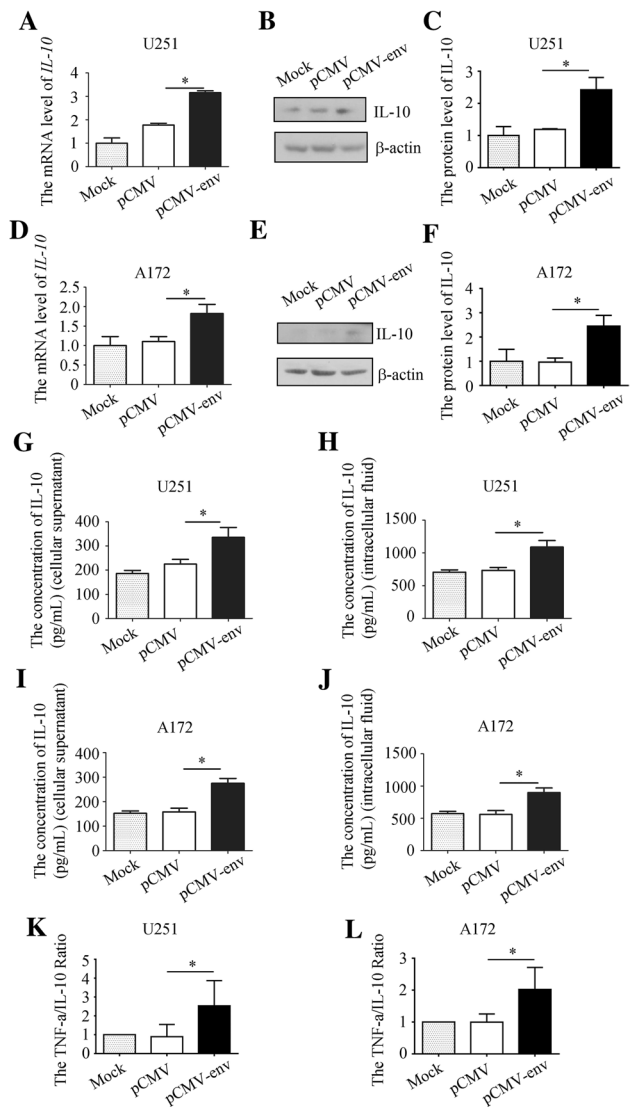


Fig. 2 HERV-W env increases the expression of IL-10 in U251 and A172 cells. (A and D) mRNA expression levels of IL-10 after overexpression of HERV-W env in U251 and A172 cells. (B and C) A representative western blot and the protein expression level of IL-10 after overexpression of HERV-W env in U251 cells. (E and F) A representative western blot and the protein expression level of IL-10 after transfection of A172 cells with HERV-W env. (G and H) Expression levels of IL-10 in the culture supernatant and intracellular fluid of U251 cells, measured by ELISA. (I and J) Expression levels of IL-10 in the culture supernatant and intracellular fluid of A172 cells, measured by ELISA. (K and L) The TNF- α /IL-10 ratio in the supernatants of U251 and A172 cells after overexpression of HERV-W env, measured by ELISA. The data represent the mean \pm standard deviation of three independent experiments. *, $P < 0.05$ compared to the pCMV control

results showed that HERV-W env significantly increased the mRNA levels of TLR4 in U251 and A172 cells, by 1.6- and 1.8-fold, respectively (Fig. 3A and D). HERV-W env expression also resulted in increased protein levels of TLR4 in U251 and A172 cells (Fig. 3B, C, E, and F). Furthermore,

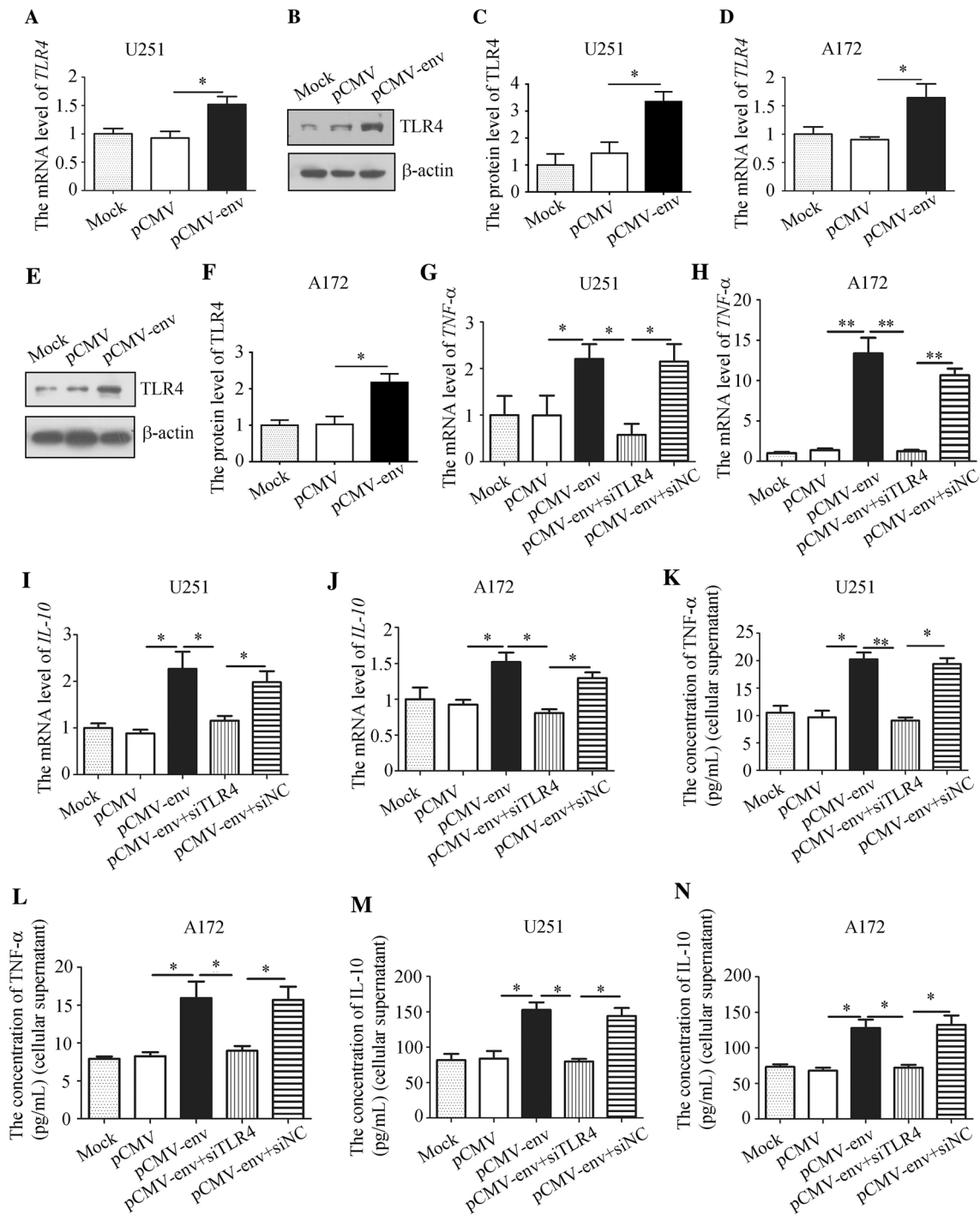


Fig. 3 HERV-W env increases the expression of TLR4 in U251 and A172 cells. (A and D) mRNA expression levels of TLR4 in U251 and A172 cells after overexpression of HERV-W env. (B and C) representative western blot and the protein expression level of TLR4 after overexpression of HERV-W env in U251 cells. (E and F) A representative western blot and the protein expression level of TLR4 after overexpression of HERV-W env in A172 cells. (G and H) mRNA expression levels of TNF- α in U251 and A172 cells transfected with HERV-W env and siTLR4. (I and J) mRNA expression levels of

IL-10 in U251 and A172 cells transfected with HERV-W env and siTLR4. (K and L) Expression levels of TNF- α in the culture supernatant of U251 and A172 cells transfected with HERV-W env and siTLR4, measured by ELISA. (M and N) Expression levels of IL-10 in the culture supernatant of U251 and A172 cells transfected with HERV-W env and siTLR4, measured by ELISA. The data represent the mean \pm standard deviation of three independent experiments. *, $P < 0.05$; **, $P < 0.01$ compared to pCMV, pCMV-env, or pCMV-env + siTLR4

knockdown of TLR4 decreased the mRNA levels of TNF- α and IL-10 induced by HERV-W env, but transfection with negative control siRNAs did not affect these genes (Fig. 3G–J). Meanwhile, ELISA assay showed that the knockdown of TLR4 also suppressed the secretions of TNF- α and IL-10 into the culture supernatants (Fig. 3K–N) and lysates of HERV-W-env-overexpressing cells (Fig. S3). These findings suggested that TLR4 might mediate the regulatory effect of HERV-W env on TNF- α and IL-10.

HERV-W env increases the expression of MyD88 but inhibits the expression of MyD88s in U251 and A172 cells

We then investigated the effect of HERV-W env on the downstream signaling pathway of TLR4. The results indicated that HERV-W env increased the mRNA levels of MyD88, a downstream target of TLR4, by 4.4- and 1.8-fold in U251 and A172 cells, respectively (Fig. 4A and B). MyD88s, a splice variant of MyD88, functions as a negative regulator of the TLR4 signal [43]. The data showed that the mRNA level of MyD88s decreased by 72% when HERV-W env was overexpressed in U251 cells (Fig. 4C). Consistently, the mRNA level of MyD88s also decreased by 70% in A172 cells after overexpression of HERV-W env (Fig. 4D).

MyD88s suppresses the expression of TNF- α and IL-10 induced by HERV-W env in U251 and 1127 cells

To further investigate the role of MyD88s in HERV-W-env-induced inflammation, U251 and A172 cells were transfected with the plasmid pCMV-Myc-MyD88s. The over-expression of MyD88s is shown in Fig. S4. Compared with pCMV-env-transfected cells, Myd88s decreased the mRNA levels of HERV-W-env-induced TNF- α by 85% and 90%, respectively, in U251 and A172 cells (Fig. 4E and F). Meanwhile, Myd88s also decreased the mRNA levels of IL-10 induced by HERV-W env to 21% and 42% in U251 and A172 cells, respectively (Fig. 5A and B). Consistent with this, ELISA results showed that MyD88s overexpression reduced the protein levels of TNF- α in the culture supernatant and lysates of U251 and A172 cells (Fig. 4G–J). Similarly, MyD88s also decreased the secretion of IL-10 in these cells (Fig. 5C–F). The significant difference between cells transfected with pCMV-env + pEGFP and cells transfected with pCMV-env + MyD88s suggested a specific regulatory role of MyD88s in HERV-W-env-induced TNF- α and IL-10 expression. These results indicated that MyD88s might downregulate the expressions of IL-10 and TNF- α induced by HERV-W env.

In summary, the results of this study suggest that HERV-W env promotes the production of TNF- α and IL-10 through the TLR4/MyD88 cascade by inhibiting MyD88s.

Discussion

Neuroinflammation is a crucial pathogenic mechanism contributing to several neuropsychiatric disorders, such as MS and schizophrenia. Accumulating evidence suggests that HERV-W env is a potential pathogenic factor in neuroinflammation. Our previous studies have shown that HERV-W env can boost nitric oxide levels [8] and elicit a strong cytotoxic T lymphocyte response [11]. We have also discovered that HERV-W env can elevate C-reactive protein (CRP) expression, leading to an inflammatory response in the CNS [44, 45]. In this study, we showed that HERV-W env overexpression increased the level of TNF- α , one of the inflammatory cytokines, in glial cells. Interestingly, we also found that HERV-W env increased the level of IL-10. IL-10 is an essential cytokine with anti-inflammatory properties that suppresses the production of several pro-inflammatory cytokines [25, 26]. This suggests that HERV-W env affects the inflammatory balance in glial cells. IL-10 is a potential pathogenic gene involved in several neuropsychiatric disorders, such as bipolar disorder and schizophrenia [25, 26]. Hence, the study of HERV-W-env-induced IL-10 might indicate a possible pathogenic mechanism of HERV-W env in neuropsychiatric disorders.

Increasing evidence shows that TNF- α is an essential regulatory target of IL-10. In monocytes, macrophages, and neutrophilic granulocytes, IL-10 can effectively inhibit TNF- α production [46–48]. Although IL-10 is considered an anti-inflammatory cytokine and can inhibit the secretion of TNF- α , several studies have identified other functions of IL-10 [48–50]. For example, IL-10 stimulates the cytotoxic activity of NK cells and enhances the IL-2-induced production of TNF- α in these cells [51]. IL-10 can cooperate with TNF- α to activate HIV-1 from latently and acutely infected monocyte/macrophage cells and T cells [52–54]. Furthermore, clinical studies have demonstrated simultaneous increases in IL-10 and TNF- α in neuropsychiatric disorders [25]. Here, we found that HERV-W env enhanced TNF- α expression and concurrently upregulated TNF- α and IL-10 in glial cells. Thus, IL-10 might cooperate with TNF- α to mediate the pathogenic role of HERV-W env in neuropsychiatric disorders.

TNF- α and IL-10 maintain the intracellular balance of pro- and anti-inflammatory responses. The TNF- α /IL-10 ratio is a vital inflammatory marker that reflects the level of systemic inflammation [39, 40]. Clinical studies have shown an increased plasma TNF- α /IL-10 ratio in periodontitis patients [55]. Our results suggested that HERV-W env might disrupt the pro-inflammatory/anti-inflammatory balance in the nervous system. The high ratio of secreted TNF- α /IL-10 might also contribute to a systemic pro-inflammatory state in the circulating peripheral blood.

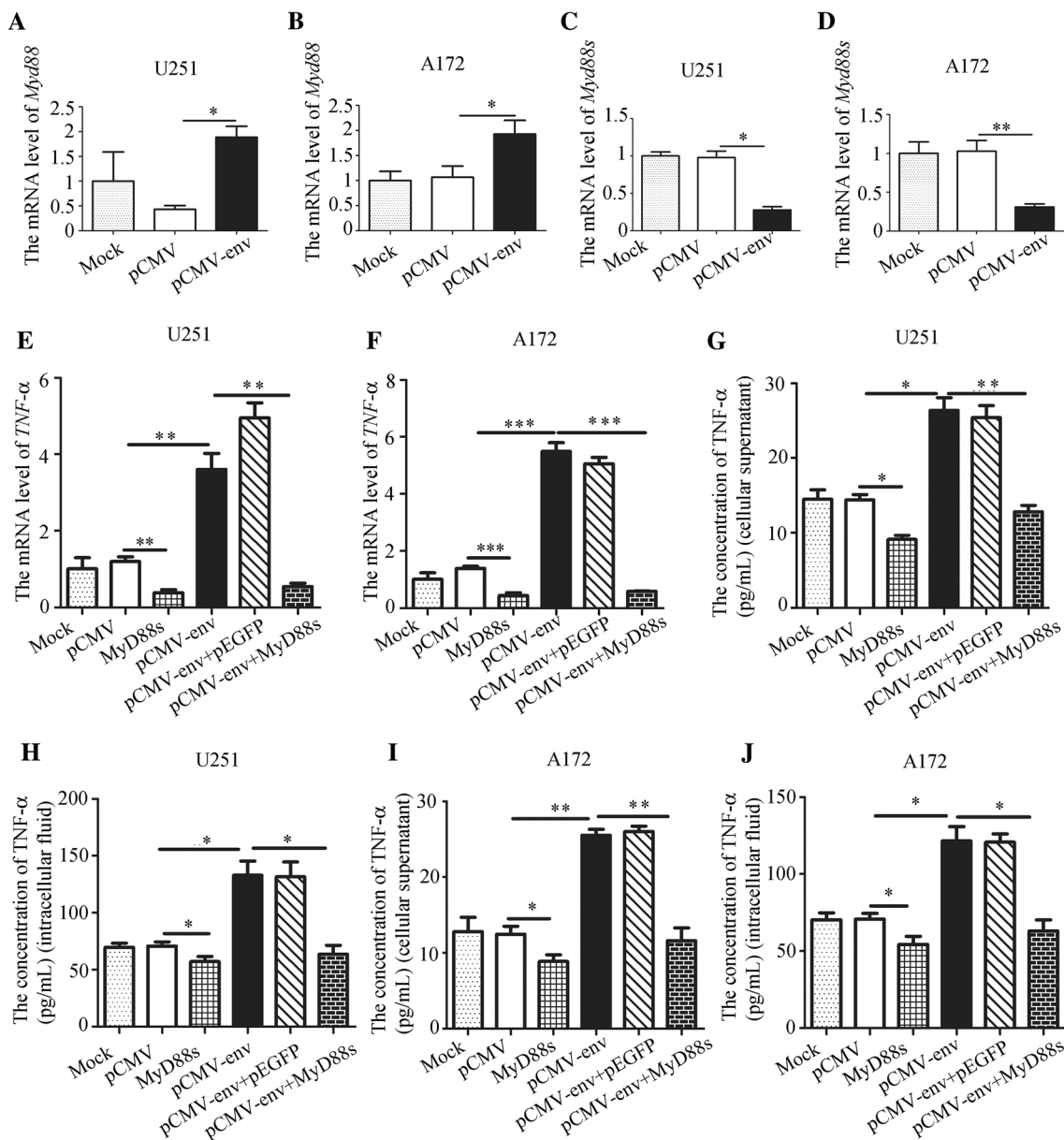


Fig. 4 HERV-W env inhibits the expression of MyD88s, and MyD88s suppresses the expression of TNF- α induced by HERV-W env in U251 and A172 cells. (A and B) mRNA expression levels of Myd88 in U251 and A172 cells transfected with HERV-W env. (C and D) mRNA expression levels of Myd88s in U251 and A172 cells after transfection with HERV-W env. (E and F) mRNA expression levels of TNF- α in U251 and A172 cells transfected with HERV-W env and MyD88s. (G and H) Expression levels of TNF- α in the culture super-

natant and intracellular fluid of U251 cells transfected with HERV-W env and MyD88s, measured by ELISA assay. (I and J) Expression levels of TNF- α in the culture supernatant and lysates of A172 cells transfected with HERV-W env and MyD88s, measured by ELISA. Data represent the mean \pm standard deviation of three independent experiments. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ compared to pCMV or pCMV-env

TLRs are a type of PRRs that play a pivotal role in the production of inflammation-related cytokines [56]. In recent years, it has been established that TLRs may be a vital mediator of the inflammatory reaction induced by HERV-W env in the nervous system [57, 58]. In our previous work, we discover a HERV-W env/TLR3/CRP signaling pathway in glial cells, in which TLR3 functions as a receptor of HERV-W

env [44]. Other studies have suggested that HERV-W env is also a highly potent TLR4 agonist of endogenous origin and induces TLR4-dependent pro-inflammatory stimulation of immune cells *in vitro* and *in vivo* [14, 15, 30]. As expected, in this work, we also found a robust regulatory effect of HERV-W env on TLR4 at both the mRNA and protein levels in human glial cells, further supporting this assumption. We

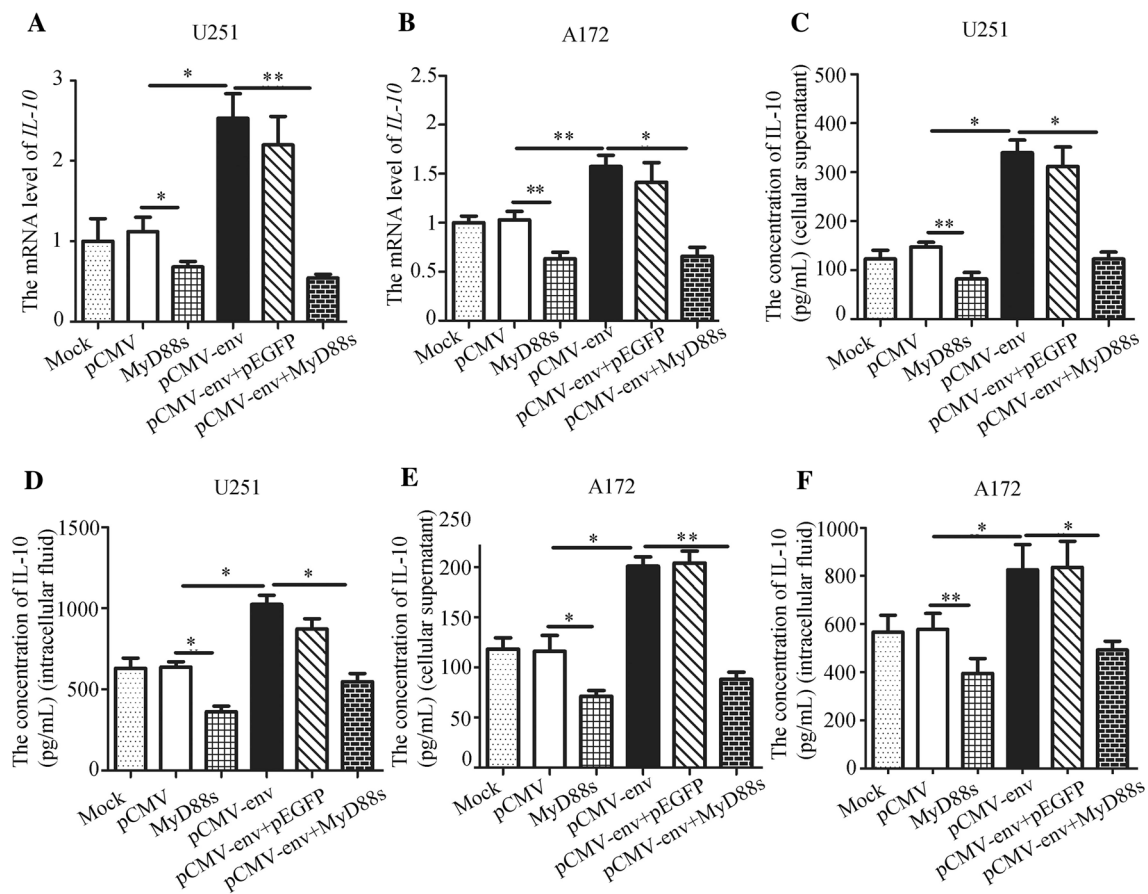


Fig. 5 MyD88s suppresses the expression of IL-10 induced by HERV-W env in U251 and A172 cells. (A and B) mRNA expression levels of IL-10 in U251 and A172 cells transfected with HERV-W env and MyD88s. (C and D) Expression levels of IL-10 in the culture supernatant and lysates of U251 cells transfected with HERV-W env

and MyD88s, measured by ELISA. (E and F) Expression levels of IL-10 in the culture supernatant and lysates of A172 cells transfected with HERV-W env and MyD88s, measured by ELISA. Data represent the mean \pm standard deviation of three independent experiments. *, $P < 0.05$; **, $P < 0.01$ compared to pCMV or pCMV-env

also found that TLR4 deficiency effectively impaired the expressions of the HERV-W-env-induced cytokines TNF- α and IL-10, suggesting that TLR4 might be a pivotal molecule in the regulation of TNF- α and IL-10 by HERV-W env. TLR3 and TLR4 may be essential factors in the molecular mechanisms by which HERV-W env regulates inflammation in neuropsychological diseases, triggering an innate immune reaction. Therefore, different TLRs may respond to HERV-W env in various tissues or fluids, functioning as mediators to induce inflammation.

Previous findings have suggested a potential role of TLR4 in the inflammatory effect of HERV-W env. However, to the best of our knowledge, no specific downstream pathways induced by HERV-W env through TLR4 have been reported. The MyD88-dependent mechanism is one of the most critical signal pathways downstream of TLR4. The TLR4 cascade occurs via the adaptor molecule MyD88 and evokes a pro-inflammatory cytokine response by regulating the activation of transcription factor NF- κ B [59, 60]. Indeed, in this

study, we found that HERV-W env elevated the expression of MyD88 in human glioma cells, suggesting that MyD88 was a potential downstream target in the HERV-W env-TLR4 signal cascade.

In contrast, MyD88s, a splice variant of MyD88 that lacks the INT domain, acts as a dominant-negative form of MyD88 [61]. MyD88s can compete with full-length MyD88 for receptor binding and inhibit the downstream signaling pathway of MyD88 [62]. In monocytes, MyD88s inhibits the induction of TNF- α [43, 63]. More interestingly, we found that HERV-W env could downregulate the expression of MyD88s in U251 and A172 cells. Furthermore, MyD88s overexpression suppressed the mRNA and protein expressions of IL-10 and TNF- α induced by HERV-W env. These results indicated that inhibiting the negative regulator MyD88s might be a possible mechanism for HERV-W env to activate the TLR4/MyD88 downstream pathway. Collectively, our findings suggested the existence of a HERV-W env-TLR4-MyD88 cascade signaling pathway

and a Myd88s-dependent negative regulatory mechanism for inducing the secretions of the cytokines TNF- α and IL-10 in glial cells. However, more work still needs to be done to explore how HERV-W env impairs the generation of MyD88s.

Recent studies have suggested that HERV-W env is a potential pathogenic factor in neuropsychological diseases, but the specific molecular mechanisms involved are still unclear. Our results provided evidence of the pro-inflammatory potential of HERV-W env in the nervous system. We also found that HERV-W env induced TNF- α and IL-10 expressions simultaneously and that the TLR4/MyD88 pathway played a vital role in triggering neuroinflammation. These findings will lead to a better understanding of neuroinflammation, a common thread in neurological diseases.

In conclusion, we observed a regulatory effect of HERV-W env on the expressions of the inflammation-related cytokines IL-10 and TNF- α in glial cells that involves the downregulation of MyD88s. These data strengthen the evidence that HERV-W env has an inflammation-regulatory function and provide further evidence for the immunomodulatory properties of HERV-W env in the nervous system.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human or animal subjects performed by any of the authors.

References

- Vargiu L, Rodriguez-Tome P, Sperber GO, Cadeddu M, Grandi N, Blikstad V, Tramontano E, Blomberg J (2016) Classification and characterization of human endogenous retroviruses; mosaic forms are common. *Retrovirology* 13:7. <https://doi.org/10.1186/s12977-015-0232-y>
- Johnson WE (2019) Origins and evolutionary consequences of ancient endogenous retroviruses. *Nat Rev Microbiol* 17(6):355–370. <https://doi.org/10.1038/s41579-019-0189-2>
- Fu B, Ma H, Liu D (2019) Endogenous retroviruses function as gene expression regulatory elements during mammalian pre-implantation embryo development. *Int J Mol Sci*. <https://doi.org/10.3390/ijms20030790>
- Noorali S, Rotar IC, Lewis C, Pestaner JP, Pace DG, Sison A, Bagasra O (2009) Role of HERV-W syncytin-1 in placentation and maintenance of human pregnancy. *Appl Immunohistochem Mol Morphol* 17(4):319–328. <https://doi.org/10.1097/PAI.0b013e31819640f9>
- Li F, Karlsson H (2016) Expression and regulation of human endogenous retrovirus W elements. *APMIS* 124(1–2):52–66. <https://doi.org/10.1111/apm.12478>
- Huang WJ, Liu ZC, Wei W, Wang GH, Wu JG, Zhu F (2006) Human endogenous retroviral pol RNA and protein detected and identified in the blood of individuals with schizophrenia. *Schizophr Res* 83(2–3):193–199. <https://doi.org/10.1016/j.schres.2006.01.007>
- Chen Y, Yan Q, Zhou P, Li S, Zhu F (2019) HERV-W env regulates calcium influx via activating TRPC3 channel together with depressing DISC1 in human neuroblastoma cells. *J Neurovirol* 25(1):101–113. <https://doi.org/10.1007/s13365-018-0692-7>
- Perron H, Mekaoui L, Bernard C, Veas F, Stefan I, Leboyer M (2008) Endogenous retrovirus type W GAG and envelope protein antigenemia in serum of schizophrenic patients. *Biol Psychiatry* 64(12):1019–1023. <https://doi.org/10.1016/j.biopsych.2008.06.028>
- Qin C, Li S, Yan Q, Wang X, Chen Y, Zhou P, Lu M, Zhu F (2016) Elevation of Ser9 phosphorylation of GSK3 β is required for HERV-W env-mediated BDNF signaling in human U251 cells. *Neurosci Lett* 627:84–91. <https://doi.org/10.1016/j.neulet.2016.05.036>
- Xiao R, Li S, Cao Q, Wang X, Yan Q, Tu X, Zhu Y, Zhu F (2017) Human endogenous retrovirus W env increases nitric oxide production and enhances the migration ability of microglia by regulating the expression of inducible nitric oxide synthase. *Virology* 522(3):216–225. <https://doi.org/10.1016/j.virus.2017.07.017>
- Tu X, Li S, Zhao L, Xiao R, Wang X, Zhu F (2017) Human leukemia antigen-A*0201-restricted epitopes of human endogenous retrovirus W family envelope (HERV-W env) induce strong cytotoxic T lymphocyte responses. *Virology* 522(4):280–289. <https://doi.org/10.1016/j.virus.2017.07.017>
- Dolei A, Uleri E, Ibba G, Caocci M, Piu C, Serra C (2015) The aliens inside human DNA: HERV-W/MSRV/syncytin-1 endogenous retroviruses and neurodegeneration. *J Infect Dev Ctries* 9(6):577–587. <https://doi.org/10.3855/jidc.6916>
- Kremer D, Schichel T, Forster M, Tzekova N, Bernard C, van der Valk P, van Horssen J, Hartung HP, Perron H, Kury P (2013) Human endogenous retrovirus type W envelope protein inhibits oligodendroglial precursor cell differentiation. *Ann Neurol* 74(5):721–732. <https://doi.org/10.1002/ana.23970>
- Rolland A, Jouvin-Marche E, Saresella M, Ferrante P, Cavaretta R, Creange A, Marche P, Perron H (2005) Correlation between disease severity and in vitro cytokine production mediated by MSRV (multiple sclerosis associated retroviral element) envelope protein in patients with multiple sclerosis. *J Neuroimmunol* 160(1–2):195–203. <https://doi.org/10.1016/j.jneuroim.2004.10.019>
- Rolland A, Jouvin-Marche E, Viret C, Faure M, Perron H, Marche PN (2006) The envelope protein of a human endogenous retrovirus-W family activates innate immunity through CD14/TLR4 and promotes Th1-like responses. *J Immunol* 176(12):7636–7644. <https://doi.org/10.4049/jimmunol.176.12.7636>
- Bandil K, Singhal P, Dogra A, Rawal SK, Doval DC, Varshney AK, Bharadwaj M (2017) Association of SNPs/haplotypes in promoter of TNF A and IL-10 gene together with life style factors in prostate cancer progression in Indian population. *Inflamm Res* 66(12):1085–1097. <https://doi.org/10.1007/s00011-017-1088-5>
- Gomes CP, Torloni MR, Gueuvoghlian-Silva BY, Alexandre SM, Mattar R, Daher S (2013) Cytokine levels in gestational diabetes mellitus: a systematic review of the literature. *Am J Reprod Immunol* 69(6):545–557. <https://doi.org/10.1111/aji.12088>

18. Hassan MO, Dix-Peek T, Duarte R, Dickens C, Naidoo S, Vachiat A, Grinter S, Manga P, Naicker S (2020) Association of chronic inflammation and accelerated atherosclerosis among an indigenous black population with chronic kidney disease. *PLoS One* 15(7):e0232741. <https://doi.org/10.1371/journal.pone.0232741>
19. Sag S, Sag MS, Tekeoglu I, Kamanli A, Nas K, Acar BA (2020) Central nervous system involvement in rheumatoid arthritis: possible role of chronic inflammation and tnf blocker therapy. *Acta Neurol Belgica* 120(1):25–31. <https://doi.org/10.1007/s13760-017-0879-3>
20. Brostjan C, Oehler R (2020) The role of neutrophil death in chronic inflammation and cancer. *Cell Death Discov* 6:26. <https://doi.org/10.1038/s41420-020-0255-6>
21. Neurath MF (2020) IL-36 in chronic inflammation and cancer. *Cytokine Growth Fact Rev*. <https://doi.org/10.1016/j.cytogfr.2020.06.006>
22. Potvin S, Stip E, Sepehry AA, Gendron A, Bah R, Kouassi E (2008) Inflammatory cytokine alterations in schizophrenia: a systematic quantitative review. *Biol Psychiatry* 63(8):801–808. <https://doi.org/10.1016/j.biopsych.2007.09.024>
23. Shen SJ, Shui SF, Xiao BK, Yang JY, Huang RQ (2017) Anti-inflammation effect of Jinlingzi San in rat metabonomics based on 1H-NMR and LC-MS technology. *Zhongguo Zhong Yao Za Zhi* 42(2):363–369. <https://doi.org/10.19540/j.cnki.cjcm.20161222.028>
24. Zhang W, Chen H (2002) The study on the interleukin-8 (IL-8). *Sheng Wu Yi Xue Gong Cheng Xue Za Zhi* 19(4):697–702
25. Kunz M, Cereser KM, Goi PD, Fries GR, Teixeira AL, Fernandes BS, Belmonte-de-Abreu PS, Kauer-Sant'Anna M, Kapczinski F, Gama CS (2011) Serum levels of IL-6, IL-10 and TNF-alpha in patients with bipolar disorder and schizophrenia: differences in pro- and anti-inflammatory balance. *Rev Bras Psiquiatr* 33(3):268–274
26. Bocchio Chiavetto L, Boin F, Zanardini R, Popoli M, Michelato A, Bignotti S, Tura GB, Gennarelli M (2002) Association between promoter polymorphic haplotypes of interleukin-10 gene and schizophrenia. *Biol Psychiatry* 51(6):480–484
27. Magliozzi R, Howell OW, Nicholas R, Cruciani C, Castellaro M, Romualdi C, Rossi S, Pitteri M, Benedetti MD, Gajofatto A, Pizzini FB, Montemezzi S, Rasia S, Capra R, Bertoldo A, Facchiano F, Monaco S, Reynolds R, Calabrese M (2018) Inflammatory intrathecal profiles and cortical damage in multiple sclerosis. *Ann Neurol* 83(4):739–755. <https://doi.org/10.1002/ana.25197>
28. Muller N, Myint AM, Schwarz MJ (2012) Inflammation in schizophrenia. *Adv Protein Chem Struct Biol* 88:49–68. <https://doi.org/10.1016/B978-0-12-398314-5.00003-9>
29. Brubaker SW, Bonham KS, Zanoni I, Kagan JC (2015) Innate immune pattern recognition: a cell biological perspective. *Annu Rev Immunol* 33:257–290. <https://doi.org/10.1146/annurev-immunol-032414-112240>
30. Duperray A, Barbe D, Raguenez G, Weksler BB, Romero IA, Couraud PO, Perron H, Marche PN (2015) Inflammatory response of endothelial cells to a human endogenous retrovirus associated with multiple sclerosis is mediated by TLR4. *Int Immunol* 27(11):545–553. <https://doi.org/10.1093/intimm/dxv025>
31. Perron H, Dougier-Reynaud HL, Lomparski C, Popa I, Firouzi R, Bertrand JB, Marusic S, Portoukalian J, Jouvin-Marche E, Villiers CL, Touraine JL, Marche PN (2013) Human endogenous retrovirus protein activates innate immunity and promotes experimental allergic encephalomyelitis in mice. *PLoS One* 8(12):e80128. <https://doi.org/10.1371/journal.pone.0080128>
32. Kuzmich NN, Sivak KV, Chubarev VN, Porozov YB, Savateeva-Lyubimova TN, Peri F (2017) TLR4 signaling pathway modulators as potential therapeutics in inflammation and sepsis. *Vaccines (Basel)*. <https://doi.org/10.3390/vaccines5040034>
33. Feng Z, Li Q, Meng R, Yi B, Xu Q (2018) METTL3 regulates alternative splicing of MyD88 upon the lipopolysaccharide-induced inflammatory response in human dental pulp cells. *J Cell Mol Med* 22(5):2558–2568. <https://doi.org/10.1111/jcmm.13491>
34. Janssens S, Burns K, Vercammen E, Tschopp J, Beyaert R (2003) MyD88S, a splice variant of MyD88, differentially modulates NF-kappaB- and AP-1-dependent gene expression. *FEBS Lett* 548(1–3):103–107
35. Huang W, Li S, Hu Y, Yu H, Luo F, Zhang Q, Zhu F (2011) Implication of the env gene of the human endogenous retrovirus W family in the expression of BDNF and DRD3 and development of recent-onset schizophrenia. *Schizophr Bull* 37(5):988–1000. <https://doi.org/10.1093/schbul/sbp166>
36. Liu Y, Liu L, Zhou Y, Zhou P, Yan Q, Chen X, Ding S, Zhu F (2019) CKLF1 enhances inflammation-mediated carcinogenesis and prevents doxorubicin-induced apoptosis via IL6/STAT3 signaling in HCC. *Clin Cancer Res* 25(13):4141–4154. <https://doi.org/10.1158/1078-0432.CCR-18-3510>
37. Zeuner MT, Vallance T, Vaiyapuri S, Cottrell GS, Wiedera D (2017) Development and characterisation of a novel NF-kappaB reporter cell line for investigation of neuroinflammation. *Mediat Inflamm* 2017:6209865. <https://doi.org/10.1155/2017/6209865>
38. Davis RL, Buck DJ, Saffarian N, Stevens CW (2007) The opioid antagonist, beta-funaltrexamine, inhibits chemokine expression in human astroglial cells. *J Neuroimmunol* 186(1–2):141–149. <https://doi.org/10.1016/j.jneuroim.2007.03.021>
39. Azizi G, Mirshafiey A (2012) The potential role of proinflammatory and antiinflammatory cytokines in Alzheimer disease pathogenesis. *Immunopharmacol Immunotoxicol* 34(6):881–895. <https://doi.org/10.3109/08923973.2012.705292>
40. Kryvoruchko IA, Goncharova NM, Andreyeshchev SA, Yavorska TP (2015) Dynamics of changes of proinflammatory and anti-inflammatory cytokines, as well as some indices of the blood peroxidation system, in the patients with various pancreatic pseudocyst types. *Klinichna Khirurgiia* 4:13–18
41. Lee MB, Lee JH, Hong SH, You JS, Nam ST, Kim HW, Park YH, Lee D, Min KY, Park YM, Kim YM, Kim HS, Choi WS (2017) JQ1, a BET inhibitor, controls TLR4-induced IL-10 production in regulatory B cells by BRD4-NF-kappaB axis. *BMB Rep* 50(12):640–646. <https://doi.org/10.5483/bmbrep.2017.50.12.194>
42. Takbiri Osgoei L, Parivar K, Ebrahimi M, Mortaz E (2018) Nicotine modulates the release of inflammatory cytokines and expression of TLR2, TLR4 of cord blood mononuclear cells. *Iran J Allergy Asthma Immunol* 17(4):372–378. <https://doi.org/10.18502/ijaai.v17i4.96>
43. Adib-Conquy M, Adrie C, Fitting C, Gattolliat O, Beyaert R, Cavaillon JM (2006) Up-regulation of MyD88s and SIGIRR, molecules inhibiting Toll-like receptor signaling, in monocytes from septic patients. *Crit Care Med* 34(9):2377–2385. <https://doi.org/10.1097/01.CCM.0000233875.93866.88>
44. Wang X, Liu Z, Wang P, Li S, Zeng J, Tu X, Yan Q, Xiao Z, Pan M, Zhu F (2018) Syncytin-1, an endogenous retroviral protein, triggers the activation of CRP via TLR3 signal cascade in glial cells. *Brain Behav Immun* 67:324–334. <https://doi.org/10.1016/j.bbi.2017.09.009>
45. Wang X, Huang J, Zhu F (2018) Human endogenous retroviral envelope protein syncytin-1 and inflammatory abnormalities in neuropsychological diseases. *Front Psychiatry* 9:422. <https://doi.org/10.3389/fpsy.2018.00422>
46. de Waal Malefyt R, Abrams J, Bennett B, Figdor CG, de Vries JE (1991) Interleukin 10(IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes. *J Exp Med* 174(5):1209–1220. <https://doi.org/10.1084/jem.174.5.1209>

47. Fiorentino DF, Zlotnik A, Mosmann TR, Howard M, O'Garra A (1991) IL-10 inhibits cytokine production by activated macrophages. *J Immunol* 147(11):3815–3822
48. Sabat R, Grutz G, Warszawska K, Kirsch S, Witte E, Wolk K, Geginat J (2010) Biology of interleukin-10. *Cytokine Growth Fact Rev* 21(5):331–344. <https://doi.org/10.1016/j.cytogfr.2010.09.002>
49. Burdin N, Rousset F, Banchereau J (1997) B-cell-derived IL-10: production and function. *Methods* 11(1):98–111. <https://doi.org/10.1006/meth.1996.0393>
50. Levy Y, Brouet JC (1994) Interleukin-10 prevents spontaneous death of germinal center B cells by induction of the bcl-2 protein. *J Clin Investig* 93(1):424–428. <https://doi.org/10.1172/JCI116977>
51. Carson WE, Lindemann MJ, Baiocchi R, Linett M, Tan JC, Chou CC, Narula S, Caligiuri MA (1995) The functional characterization of interleukin-10 receptor expression on human natural killer cells. *Blood* 85(12):3577–3585
52. Iribarren P, Chen K, Gong W, Cho EH, Lockett S, Uranchimeg B, Wang JM (2007) Interleukin 10 and TNF α synergistically enhance the expression of the G protein-coupled formylpeptide receptor 2 in microglia. *Neurobiol Dis* 27(1):90–98. <https://doi.org/10.1016/j.nbd.2007.04.010>
53. Finnegan A, Roebuck KA, Nakai BE, Gu DS, Rabbi MF, Song S, Landay AL (1996) IL-10 cooperates with TNF- α to activate HIV-1 from latently and acutely infected cells of monocyte/macrophage lineage. *J Immunol* 156(2):841–851
54. Rabbi MF, Finnegan A, Al-Harathi L, Song S, Roebuck KA (1998) Interleukin-10 enhances tumor necrosis factor- α activation of HIV-1 transcription in latently infected T cells. *J Acquir Immune Def Syndrom Hum Retrovirol* 19(4):321–331. <https://doi.org/10.1097/00042560-199812010-00002>
55. Purnamasari D, Khumaedi AI, Soerосо Y, Marhamah S (2019) The influence of diabetes and or periodontitis on inflammation and adiponectin level. *Diabetes Metabol Syndrome* 13(3):2176–2182. <https://doi.org/10.1016/j.dsx.2019.05.012>
56. West AP, Brodsky IE, Rahner C, Woo DK, Erdjument-Bromage H, Tempst P, Walsh MC, Choi Y, Shadel GS, Ghosh S (2011) TLR signalling augments macrophage bactericidal activity through mitochondrial ROS. *Nature* 472(7344):476–480. <https://doi.org/10.1038/nature09973>
57. Madeira A, Burgelin I, Perron H, Curtin F, Lang AB, Faucard R (2016) MSRV envelope protein is a potent, endogenous and pathogenic agonist of human toll-like receptor 4: Relevance of GNbAC1 in multiple sclerosis treatment. *J Neuroimmunol* 291:29–38. <https://doi.org/10.1016/j.jneuroim.2015.12.006>
58. Perron H, Lang A (2010) The human endogenous retrovirus link between genes and environment in multiple sclerosis and in multifactorial diseases associating neuroinflammation. *Clin Rev Allergy Immunol* 39(1):51–61. <https://doi.org/10.1007/s12016-009-8170-x>
59. Weber ANR, Cardona Gloria Y, Cinar O, Reinhardt HC, Pezzutto A, Wolz OO (2018) Oncogenic MYD88 mutations in lymphoma: novel insights and therapeutic possibilities. *Cancer Immunol Immunother* 67(11):1797–1807. <https://doi.org/10.1007/s00262-018-2242-9>
60. Zhu M, Yu K, Wang L, Yu S (2018) Contribution of drugs acting on the TLRs/MyD88 signaling pathway on colitis-associated cancer. *Pharmazie* 73(7):363–368. <https://doi.org/10.1691/ph.2018.8388>
61. De Arras L, Alper S (2013) Limiting of the innate immune response by SF3A-dependent control of MyD88 alternative mRNA splicing. *PLoS Genet* 9(10):e1003855. <https://doi.org/10.1371/journal.pgen.1003855>
62. Vickers TA, Zhang H, Graham MJ, Lemonidis KM, Zhao C, Dean NM (2006) Modification of MyD88 mRNA splicing and inhibition of IL-1 β signaling in cell culture and in mice with a 2'-O-methoxyethyl-modified oligonucleotide. *J Immunol* 176(6):3652–3661. <https://doi.org/10.4049/jimmunol.176.6.3652>
63. Janssens S, Burns K, Tschopp J, Beyaert R (2002) Regulation of interleukin-1- and lipopolysaccharide-induced NF- κ B activation by alternative splicing of MyD88. *CB* 12(6):467–471. [https://doi.org/10.1016/s0960-9822\(02\)00712-1](https://doi.org/10.1016/s0960-9822(02)00712-1)

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