Published in final edited form as:

J Neuroimmune Pharmacol. 2016 September ; 11(3): 562–571. doi:10.1007/s11481-016-9679-4.

Modulation effect of HIV-1 viral proteins and nicotine on expression of the immune-related genes in brain of the HIV-1 transgenic rats

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Abstract

The human immunodeficiency virus-1 transgenic (HIV-1Tg) rat is a non-infectious rodent model for HIV-1 infection which develops altered immune-responses similar to those in persons infected with HIV-1. HIV-1Tg and F344 rats respond significantly different to morphine, ethanol, nicotine and other psychostimulants, although the molecular mechanisms underlying these differences remain largely undetermined. Here, we compared expression of 52 immune-related genes in the prefrontal cortex (PFC), nucleus accumbens (NAc), and ventral tegmental area (VTA) of HIV-1Tg and F344 rats treated with either nicotine (0.4 mg/kg nicotine, base, *s.c.*) or saline for 27 days, to identify differentially expressed genes in the presence of HIV-1 with and without nicotine treatment. Using quantitative RT-PCR array, we measured RNA expression levels. Results showed that RNA expression of CASP3, CCL5, CX3CL1, CX3CR1, IL1 α , LRF4, LFR7, TGF β 1 and TLR4 in NAc, CCL2, CCL5, TGF β 1 and TLR4 in PFC, and CASP3, CX3CR1, IFN α 1, IL1 β and IL6 in VTA was significantly modulated in HIV-1Tg rats compared with F344 rats. IL1 α showed a 58% ($P = 0.000072$) decrease and IRF6 showed a 93.7% increase ($P = 0.000227$) in the NAc of HIV-1Tg compared with F344 rats; results remained significant after correction for multiple testing. We also found that several genes were significantly modulated by nicotine in HIV-1Tg rats while only a small number of immune-related genes were altered by nicotine in F344 rats. These findings imply that HIV-1 viral proteins greatly impact immune function and alter responsiveness to nicotine in certain immune-related genes.

Keywords

nicotine; HIV-1 transgenic rat; CNS immunity; prefrontal cortex; nucleus accumbens; ventral tegmental area

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Financial Disclosures: The authors claim no conflict interest regarding this report.

Introduction

HIV-1 infection involves the actions of HIV-1 viral proteins on targeted cells of the immune system, such as macrophages and T-lymphocytes (Cicala et al., 2002, Verani et al., 2005, Jiang and Aiken, 2006). Like the peripheral immune system, the central nervous system (CNS) is also highly vulnerable to HIV-1 infection. The brain regions targeted by HIV-1 virus include the prefrontal cortex region (PFC), nucleus accumbens (NAc), and ventral tegmental area (VTA) (Midde et al., 2011, Nesil et al., 2015). Although combination anti-retroviral therapy (cART) leads to a temporary reversal of HIV dementia (Portegies, 1995, Gisslen et al., 1998, Gendelman et al., 1998), both HIV-associated neurocognitive disorders (HAND) and HIV-associated dementia (HAD) are resistant to cART and become more severe as the lifespan of HIV-1 infected patients is prolonged (McArthur et al., 2003).

To better understand the effects of HIV-1 on neurocognitive function, an HIV-1 transgenic (HIV-1Tg) rat model, which carries a *gag-pol* deleted HIV-1 genome under the control of the HIV-1 viral promoter and expresses 7 of the 9 HIV-1 genes, was developed (Reid et al., 2001). Although there is no viral replication in this animal model, viral proteins are expressed in various organs including blood (Reid et al., 2001, Mazzucchelli et al., 2004). The HIV-1Tg rat develops characteristics similar to those of humans infected with HIV-1, which include immune-response alterations, pathologies with advancing age, T-cell abnormalities and kidney failure (Ray et al., 2003, Ray et al., 2010, Ray et al., 2009). Furthermore, the HIV-1Tg rat also shows alterations in sensitivity to many psychostimulants, including morphine, alcohol and nicotine (Sarkar and Chang, 2013, Homji et al., 2012).

Since its development in 2001, the HIV-1Tg rat model has been used to study the impact of HIV-1 viral proteins on molecular, cellular, systemic, and behavioral levels. This model has gained favor in many laboratories, including ours, as a model to mimic the state of HIV-1 patients given cART. Li and colleagues discovered that several immune-related genes and pathways are altered in the brain of the HIV-1Tg rat (Li et al., 2013). Among these altered genes and pathways, many are related to cytokines such as interferon, interleukins and chemokines (Li et al., 2013). In addition, this group also found that, in HIV-1Tg rats, signaling for TLR in the PFC, interferon in the HIP, and IL-4 in the STR are significantly altered (Li et al., 2013).

It is generally accepted that, while nicotine is a substance of abuse, it also exerts positive effects in the CNS. Various studies have demonstrated nicotine's ability to modulate immune regulations (Sopori, 2002, Chang et al., 2014, Cui and Li, 2010). At a dose comparable to that achieved by "average to heavy" smokers, nicotine upregulates expression of inflammatory mediators, cytokines (IL-1 β , TNF α , and IL-18), chemokines (CCL2 and CX(3)CL1), and adhesion molecules (ICAM-1, VCAM-1, and P-selectins) in mice (Bradford et al., 2011). Pretreatment with nicotine results in upregulation of IL-8 in human gingival epithelial cells (HGECS) and this upregulation is attenuated when cells are pretreated with non-selective nicotinic acetylcholine receptor (nAChR) antagonists (Kashiwagi et al., 2012). In human lamina propria (LPT) cells, T-bet, an integral player in

coordinating immune responses, is upregulated following nicotine treatment (Kikuchi et al., 2008).

While it is clear that nicotine affects immune responses, the research is not unanimous regarding its effect on inflammation which has been implicated in the neurodegenerative effects of HIV-1 (Chang et al., 2014). For example, some studies suggest that nicotine suppresses inflammation (Nizri et al., 2009, Sugano et al., 1998, Thomsen and Mikkelsen, 2012) while others suggest that nicotine has opposite effects in cells such as aortic vascular smooth muscle cells (Wang et al., 2012). After treating SH-SY5Y cells with nicotine, Cui et al. (Cui et al., 2012) reported alterations in 14 biological pathways (Cui et al., 2012). Of the pathways significantly modulated by nicotine in SH-SY5Y cells, the Toll Like Receptor (TLR) and death receptor (DR) pathways are integral components of the innate immune system which is responsible for inflammatory responses. Further, downstream pathways of TLR and DR signaling and the expression of genes associated with innate immunity such as nuclear factor κ B (NF- κ B) were also affected by nicotine (Cui et al., 2012).

Specifically for this HIV-1Tg rat model, we found that the expression a significant number of genes related to neuroprotective function and Wnt/ β -catenin is restored by treatment with nicotine (Cao et al., 2013). More specifically, in the PFC, genes related to Wnt/ β -catenin signaling are restored to a level comparable to F344 control rats. it has been demonstrated that Wnt/ β -catenin signaling has been reported to play a major role in the early development of the nervous system and activation of this pathway has a neuroprotective effect in adults (Toledo et al., 2008). Thus, nicotine's restoration of Wnt β -catenin pathways and related genes has implications for enhanced neuronal survival in the brain of HIV-1Tg rat and treatment of various other disorders.

Further, following nicotine treatment, N-methyl-d-aspartate (NMDA) receptor function is restored, and fibroblast growth factor 9 (Fgf9) expression is increased as is Wnt5a expression which has been linked to synaptic plasticity and NMDA regulation. In the hippocampus, nicotine regulates the overexpression of a phospholipase C (Plch1) and decreases oxidative stress in HIV-1Tg rats. Nicotine has a similar effect on oxidative stress in the striatum and also restores the function of the tricarboxylic acid cycle in this brain region. This study demonstrated that nicotine has the ability to regulate a number of neurodegenerative effects of HIV-1 viral proteins in various brain regions (Cao et al., 2013).

Following from the aforementioned studies and given the fact that, despite the fact that individuals with HIV-1 are more likely to smoke cigarettes (Vigorito et al., 2015, Browning et al., 2013; Tesoriero et al., 2010), there is a lack of research investigating the potential therapeutic potential of nicotine, the current study sought, for the first time, to determine (1) the effects of HIV-1 viral proteins on the expression of immune-related genes in three brain regions which are associated with cognitive function and are known to be affected by HIV-1 and (2) the effect of nicotine on the expression of immune-related genes using the HIV-1Tg rat model.

Materials and Methods

Animals

Male HIV-1Tg rats and F344 control rats between 7 and 8 weeks were purchased from Harlan Laboratories (Indianapolis, IN). All rats were group-housed in standard plastic rat cages, maintained in a temperature-controlled environment with a 12 h light/dark cycle, and fed a standard rat diet and water *ad libitum*. All experimental procedures were conducted during the light cycle and approved by the University of Virginia Animal Care and Use Committee. Rats were divided into 4 groups with 5–6 animals per group: HIV-1Tg control; F344 control; HIV-1Tg nicotine; and F344 nicotine.

Nicotine treatment and tissue collection

Nicotine hydrogen tartrate (Sigma, St Louis, MO) was dissolved in 0.9% physiological saline solution. F344 and HIV-1Tg rats were injected subcutaneously (*s.c.*) with either saline or nicotine once per day (0.4 mg/kg/day) for 27 d before tissue collection, a procedure commonly used to study pharmacological effects of nicotine in our and other groups (Gutala et al., 2006, Matta et al., 2007, Nesil et al., 2015). The concentration of nicotine was calculated as nicotine free base, pH 7.0, according to Matta et al. (Matta et al., 2007) and based on our previous reported (Song et al., 2015, Nesil et al., 2015).

On Day 28, the animals were sacrificed and brain regions were collected. Using a rat brain matrix (Kent Scientific, Torrington, CT), 1 mm slices were taken from each brain. The slices that contained the prefrontal cortex (PFC), ventral tegmental area (VTA) and nucleus accumbens (NAc) were identified according to the rat brain atlas (Paxinos and Watson, 2005). Tissue from the regions of interest was collected from each brain using a 1.5 mm brain punch (Stoelting, Wood Dale, IL), and stored at –80°C until use.

RNA extraction and quantitative RT-PCR analysis

Total RNA was isolated using the Trizol agent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. The purity and quantity of total RNA were measured at optical densities of 260 nm and 280 nm with NanoDrop 2000c (Thermo Scientific, Waltham, MA, USA). The primers for all 52 immune-related genes were designed using Primer Express (v. 3.0) software. To avoid amplifying genomic DNA, two primers of each gene were designed to span at least one intron. A custom-designed RT-PCR array was used to measure gene expression as described previously (Cui et al., 2013, Cao et al., 2011, Wei et al., 2011). Each pair of primers and their amplicon sequences were tested using the Basic Local Alignment Search Tool (BLAST; <http://blast.ncbi.nlm.nih.gov/Blast.cgi>) to ensure the specificity of the designed primers for the targeted genes. Dissociation curves were generated to check the specificity of the primers before including them in the qRT-PCR array. All primer sequences are listed in Table 1.

Two µg of total RNA was reverse-transcribed into the first-strand cDNA using Superscript II Reverse Transcriptase. The cDNA mixture was incubated at 25°C for 10 min, 42°C for 1.5 h, and 70°C for 15 min. The PCR amplification was conducted as described previously (Cui et al., 2013, Cao et al., 2011, Wei et al., 2011). Briefly, the product was amplified in a

volume of 10 μ l containing 5.0 μ l of 2 $\times$ Power SYBR Green PCR Master Mix (Applied Biosystems) and combined sense and antisense primers (2.5 μ l; final concentration 20 nM) in a 384-well plate using the 7900HT Sequence Detection System (Applied Biosystems). The PCR conditions were as follows: 95°C for 10 min, followed by 40 cycles of 95°C for 15 sec and 60°C for 1 min. A cycle threshold was assigned at the beginning of the logarithmic phase of the amplification, and differences in the C_t values of the control and nicotine-treated groups were used to determine the relative expression of genes of interest. Melting curve analysis was applied to characterize the specificity of the amplifications.

Statistical analysis

Data are expressed as mean $\pm$ S.E.M. Expression of each gene of interest was first normalized to the expression of the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and then analyzed using a comparative C_t method (Winer et al., 1999). The relative expression of each gene was compared between the groups of HIV-1Tg control versus F344 control (to determine viral effects), HIV-1Tg nicotine versus HIV-1Tg saline (to determine the pharmacological effects of nicotine in the presence of viral proteins) and F344 nicotine versus F344 saline (to determine the pharmacological effects of nicotine in the absence of viral proteins) rats using the Student t-test. Significant alteration in mRNA expression was defined as a fold change $> 20\%$ with a P value < 0.05 . All P values given in this report are original. Bonferroni correction was used to correct for multiple testing.

Based on the literature, any difference less than 20% between two groups is less likely to be biologically significant (Wang et al., 2007, Li et al., 2004). A power analysis conducted using Power Analysis and Sample Size (PASS) software (Hintze, 2000, Cohen, 1988) revealed that a sample size of 6 is sufficient to attain at least 85% power and detect 20% expression difference between two groups using a two-tailed two-sample t-test at the 5% significance level.

Results

Effect of HIV-1 viral proteins and nicotine on expression of immune-related genes in NAc

As shown in Table 2, our qRT-PCR array analysis revealed 18 of 52 immune-related genes are significantly changed ($P < 0.05$) by HIV-1 viral proteins or nicotine in the NAc of HIV-1Tg or F344 control rats. Genes upregulated by HIV-1 include CASP3 (43%, $P=0.003$), CX3C11 (31%, $P=0.029$), LRF4 (58%, $P=0.024$), and IRF7 (95%, $P=0.00027$). Further, CCL5 (40%, $P=0.007$), CX3CR1 (27%, $P=0.012$), IL1 α (58%, $P=0.000071$), TGF β 1 (14%, $P=0.023$) and TLR4 (24%, $P=0.03$) are significantly downregulated in HIV-1Tg rats.

In the NAc of HIV-1Tg rats, nicotine significantly upregulated the expression of CCL11 (208%, $P=0.023$), CCR2 (192%, $P=0.012$) and IL1 α (69%, $P=0.006$) and downregulated expression of CX3C11 (28%, $P=0.003$), IL2 (37%, $P=0.04$), IL8R α (48%, $P=0.002$), IRF4 (32%, $P=0.041$), and TNF (48%, $P=0.044$). In F344 control rats, CASP3 (41%, $P=0.039$), IL5 (213%, $P=0.037$), IRF4 (33%, $P=0.026$) and IRF9 (41%, $P=0.002$) were upregulated following nicotine treatment and only IL1 α (36%, $P=0.023$) was downregulated. Of the genes affected, nicotine had opposite effects on IRF4 and IL1 α in the NAc of HIV-1 and

F344 rats. A comparison of the effects of HIV-1 and nicotine on IRF4 and IL1 α expression in the NAc region is given in Figure 1.

Effect of HIV-1 viral proteins and nicotine on expression of immune-related genes in PFC

Of the 52 genes examined in the PFC, we found that the expression of CASP1 (38%, $P=0.042$), CCL5 (51%, $P=0.026$), TGF β 1 (23%, $P=0.015$), and TLR4 (46%, $P=0.010$) were all downregulated and no gene was upregulated by HIV-1 viral proteins in HIV-1Tg rats (see Table 3).

Following nicotine treatment, nine genes CASP1 (34%, $P=0.006$), CCR5 (34%, $P=0.031$), CX3CR1 (28%, $P=0.035$), IL1 β (37%, $P=0.014$), II1R2 (47%, $P=0.004$), II8R α (70%, $P=0.025$), TGF β 1 (26%, $P=0.04$), TNF (47%, $P=0.036$), and TNFRSF1 α (27%, $P=0.017$) were downregulated in HIV-1Tg rats whereas only genes CCL2 (49%, $P=0.004$), II1R2 (32%, $P=0.011$), II18 (37%, $P=0.023$), TGF β 1 (34%, $P=0.006$), TLR4 (34%, $P=0.031$) and TNFRSF1 α (28%, $P=0.019$) were downregulated in F344 rats. No genes were upregulated by nicotine in the PFC of either HIV-1Tg or F344 control rats. Of the genes modulated by nicotine, only TNFRSF1 α and TGF β 1 were affected in both groups. A comparison of the effects of HIV-1 viral and nicotine on TGF β 1 and TNFRSF1 α expression in the PFC region is given in Figure 2.

Effect of HIV-1 viral proteins and nicotine on expression of immune-related genes in VTA

With the same RT-PCR technology, we also examined expression of the 52 genes in the VTA region of HIV-1Tg and F344 rats treated with nicotine or without nicotine (Table 4). Comparisons of HIV-1Tg and F344 controls revealed that HIV-1 significantly downregulates expression of II6 (74%, $P=0.000$) and upregulates expression of CASP3 (37%, $P=0.0001$), CX3CR1 (44%, $P=0.046$) and IL1 β (188%, $P=0.0005$) in the VTA region.

Nicotine treatment was found to downregulate CCR5 (70%, $P=0.005$), IL5 (58%, $P=0.036$), IRF1 (33%, $P=0.032$) and TNF (50%, $P=0.008$) expression in HIV-1Tg rats, and CASP3 (27%, $P=0.008$), CCL2 (33%, $P=0.022$) and IRF6 (42%, $P=0.02$) expression in F344 control rats. Further, IL8R α is upregulated by nicotine only in F344 rats.

Discussion

In this study, we investigated 52 immune-related genes in the three brain regions implicated in addiction and HAND with three objectives. The first objective was to determine how HIV-1 viral proteins affect expression of these immune-related genes and the second and third objectives were to determine how nicotine affects expression of those genes in the presence (i.e., in HIV-1Tg rat) and absence (i.e., F344 genetic background control rat) of viral proteins. Our results showed that HIV-1 viral proteins have a significant impact on the expression of many immune-related genes in a brain region-dependent manner (Table 5). Further, our results showed that the pharmacological effect of nicotine on expression of these immune-related genes differed in the HIV-1Tg and F344 rats, suggesting that nicotine may have different effects on expression of those immune-related genes in the presence (i.e., HIV-1Tg rats) or absence (i.e., F344 control rats) of HIV-1 viral proteins. Importantly, our data showed that treatment with nicotine appears to have the opposite effects of viral

proteins on a number of immune-related genes suggesting that nicotine could potentially reverse some of the negative effects of HIV-1 viral proteins.

Of the differentially expressed genes reported in this paper, interleukin 1 α (IL-1 α) and Interferon regulatory factor 7 (IRF7) are two representative genes that are worthy of discussion. IL1 α is a cytokine of the interleukin-1 family, which is responsible for the production of inflammation, as well as the promotion of fever and sepsis. In the NAC, we found that both HIV-1 viral proteins and nicotine reduced the expression of this gene. In the HIV-1Tg rats treated with saline, IL-1 α expression was decreased by 58% and in F344 rats treated with nicotine, IL-1 α expression was reduced by 36% compared with F344 saline controls. However, when the HIV-1Tg rats were treated with nicotine, we found the expression level of this gene was increased by 69%. These results suggest that, in the presence of HIV-1 viral proteins, nicotine works to not only reverse the suppression effect of HIV-1 viral proteins on IL-1 α expression, but also further enhanced the expression of this gene. IL-1 production is induced by endotoxins as well as cytokines such as TNF which is also associated with chronic inflammation. It has been posited that neural inflammation plays a large role in the development and progression of HIV-associated neurocognitive disorder (HAND), thus, due to its involvement in regulation of both the innate and adaptive immunity and induction of fever and host inflammation, IL-1 α regulation may be a potential target for treatment of HAND and other inflammation-related disorders.

In the case of Irf7, our results showed a 96% increase in expression in the NAc of HIV-1Tg rats. However, when treated with nicotine, HIV-1Tg rats exhibited no significant difference in NAc Irf7 expression compared to nicotine-treated F344 rats. These results suggest that nicotine suppressed the increased expression of IRF7 in the NAc resulting from HIV-1 viral proteins. Given that Irf7 is the master regulator of type I interferon production and is involved in oncogenesis and apoptosis (Ning et al., 2011), alteration of that pathway and upregulation of Irf7 suggest disturbances of the innate immune response and neuronal survival in the HIV-1Tg rat. Nicotine was able to decrease the effect of HIV-1 viral proteins on Irf7 expression, thus acting as a possible neuronal protectant.

As reported in previous studies which focused on the PFC, hippocampus, and striatum (Li et al., 2013, Cao et al., 2013), we found that HIV-1 viral proteins and nicotine affect expression of immune-related genes in a brain region-dependent manner. More specifically, we found that more genes were altered by nicotine, HIV-1 viral proteins, or the combination of both in the NAc than in the PFC or the VTA. Further, we found that no genes were up-regulated by either nicotine or HIV-1 viral proteins in the PFC, however this brain region had the highest number of significantly down-regulated genes. The VTA has the least number of genes altered by either nicotine, HIV-1 viral proteins, or the combination of the two variables. These results clearly indicate that the pathological effect of HIV-1 viral proteins and pharmacological effect of nicotine on immune-related genes differ between brain regions.

In our previous study, which was the first to perform deep-sequencing analysis of RNA transcripts associated with the HIV-1Tg rat, we discovered that three major pathways were effected by HIV-1 viral proteins; immune response-, neuronal survival-, and neurotransmission-related pathways. In the current study, we were able to expand on our

previous research which focused on identifying genes and pathways altered in the prefrontal cortex, hippocampus, and striatum of the HIV-1Tg rat (Li et al., 2013).

Although it is well documented that nicotine is an addictive substance, it also exerts neuroprotective effects in the brain. For example, nicotine treatment restored signaling in genes related to Wnt/ β -catenin to normal, restored NMDA receptor function, increased Fgf9 and Wnt5a expression in the PFC of HIV-1Tg rats (Cao et al., 2013). In the hippocampus, nicotine regulates the overexpression of phospholipase C (Plch1) and decreases oxidative stress. Nicotine has a similar effect on oxidative stress in the striatum and also restores the function of the TCA in this brain region. The effect of nicotine on oxidative stress in HIV-1Tg rats has been supported in another study recently reported by our group (Song et al., 2015). Together, these findings demonstrated that nicotine has the ability to regulate a number of neurodegenerative effects of HIV-1 viral proteins in the brain.

In addition to those *in vivo* studies, modulation of the innate immune system is also seen in a neuronal SH-SY5Y neuroblastoma cell model. After treating these cells with nicotine, Cui et al reported alterations in 14 pathways. Among these pathways are the TLR pathway and the death receptor pathway, which are integral components of the innate immune system (Cui et al., 2012). Further, downstream pathways of TLR and DR signaling and the expression of genes associated with innate immunity such as nuclear factor κ B (NF- κ B) were also affected by nicotine. These findings demonstrate that nicotine can regulate multiple innate immune-related pathways, and our data thus provide new clues to the molecular mechanisms underlying nicotine's regulatory effects on neurons in the NAc, PFC, and VTA.

In sum, this study demonstrates the HIV-1 viral proteins have significantly impacted expression of a number of immune-related genes in different brain regions. Furthermore, we found that the pharmacological effects of nicotine on expression of immune-related genes differed in the brains of HIV-1Tg rats compared with F344 control rats, suggesting that HIV-1 viral proteins can modulate the response of immune system to nicotine. Finally, we showed the biological effect of HIV-1 viral proteins and nicotine on immune-related gene expression appears to be in a brain region-dependent manner.

Acknowledgments

The authors thank Drs. Shaolin Wang, Junran Cao, and Guohua Song for their assistance with sample collection during the experiments. This work was supported, in part, by US National Institutes of Health grants DA-016149 to SLC and DA-026356 to SLC and MDL.

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Figure 1.

Regulatory effects of nicotine and HIV-1 viral proteins on IRF4 and IL1 α in the nucleus accumbens of the HIV-1Tg and F344 rats following 27 days of either saline or nicotine (0.4 mg/kg) treatment. Nicotine treatment had a significantly different effect on IRF4 and IL1 α expression in the nucleus accumbens of HIV-1Tg and F344 rats. Expression levels are reported as fold increase in mRNA levels. See Table 2 for detailed statistical analysis results (N = 5–6 animals per group).

Figure 2.

Regulatory effects of nicotine and HIV-1 viral proteins on TGFβ1 and TNFRSF1α in the prefrontal cortex of the HIV-1Tg and F344 rats following 27 days of either saline or nicotine (0.4 mg/kg) treatment. An independent-samples t-test revealed that expression of TGFβ1 and TNFRSF1α was significantly regulated in HIV-1Tg rats compared to F33 controls. Expression levels are reported as fold increase in mRNA levels. See Table 3 for detailed statistical analysis results (N = 5–6 animals per group).

Table 1

Primer sequences of immune-related genes used for quantitative RT-PCR analysis in this study

Gene Symbol	Gene Name	Forward Primer (5'-3')	Reverse Primer (5'-3')
CASP1	Caspase 1	CTCAAGTTTGGCCCTTTAGAAAT	TGAAAGTCTGTGCTGCAGATAATG
CASP3	Caspase 3	AGGCCGACTTCTCTGATGCTT	TGACCCGTCCCTTGAATTC
CCL2	Chemokine (C-C motif) ligand 2	CCCAAAGAAGCTGTAGTATTG	TCTGGACCCCAATTCCTTATTG
CCL3	Chemokine (C-C motif) ligand 3	TGAGACCAAGCCTTTTGCT	CAGATCTGCCGGTTTCTCTTG
CCL5	Chemokine (C-C motif) ligand 5	GTCTCTTTGTCTACTCTGAAGGA	GATGTATCTTGAACCCACTTCTCTC
CCL11	Chemokine (C-C motif) ligand 11	TCACGGCCACTTCTCTCAC	GGGATCTTCTTACTGTGTCATGGTAA
CCR1	Chemokine (C-C motif) receptor 1	GGGACCTTGAACCTTGAATTCATA	TGAGTCTGTGTTTCCAGAGTACTTTGT
CCR2	Chemokine (C-C motif) receptor 2	GGTCTGTACGGGGCTCAC	CCCGGGCTTCTGTCTCAT
CCR3	Chemokine (C-C motif) receptor 3	CAGTCTCTCGTATCCA	TTTAGCTCTTCTCTCTCTCAT
CCR5	Chemokine (C-C motif) receptor 5	TCATAAACACAGTGTGAAACAATTG	TCATGTTACCCACAAAACCAAAGA
CX3CL1	Chemokine (C-X3-C motif) ligand 1	CTGGCCGCGTCTCTTTCAT	TGGCACGTGATGTTGCAATT
CX3CR1	Chemokine (C-X3-C motif) receptor 1	GGGTGAGTGCTGGCACTT	GATCCAATCCGGGAAGGA
CXCL10	Chemokine (C-X-C motif) ligand 10	GGCCATAGGAAAACCTTGAAATCA	CTCTTCTCATGTTCTTTTTCATTGTG
IFN α 1	Interferon- α 1	CCTGGTGTGTGAGTACTG	GCTCTTGTTCCTGAGGTATGAG
IFN α R1	Interferon (α , β and ω) receptor 1	CAGTGCACACATCGCTTCAGA	ATTTTATTTGCCACTGTGGTCTTTAT
IFN β 1	Interferon β 1, fibroblast	GGTTCCTGCTGTGCTTCTC	TGCTAGTCTTTGTCCGAACTG
IFN γ	Interferon γ	CATTCATGAGCATCGCCAACT	TCTGATGAGTTTCAATGACAGCTTTG
IFN γ R1	Interferon γ receptor 1	CCCTTTCTCCATGATGACAGA	GCCGGGACAACTAATTGTAAGGA
IL1 α	Interleukin 1 α	GCCATAGCCCATGATTTAGAAGA	TGCTTGACGATCCTTATCAATTG
IL1 β	Interleukin 1 β	AACAGGCTCATCTGGGATCCT	TCAGGGACAGTTGCCATAGCT
IL1R1	Interleukin 1 receptor, type I	AAGTGGAAATGGTCCGAAATT	TGAAGGGTGTTCCAAAAACCTGA
IL1R2	Interleukin 1 receptor, type II	TGCACACAGGAAGGGTTTCTG	AACAGCAATCGTCTCCAAGA
IL2	Interleukin 2	TTCAATTTCCAGGCCTGAAGA	CCATGATGCTCACGTTTAAATTT
IL4	Interleukin 4	CAGACGTCTTACGGCAACA	TGCGAAGCACCTGGAA
IL5	Interleukin 5	CCATGAGCACAGTGTGAAAGA	CGTCTCATTTGCTCGTCAACAG
IL6	Interleukin 6	GCCTTTCAGGAACAGCTATGA	TGTCAACAACATCAGTCCCAAGA
IL6R	Interleukin 6 receptor	GCCCTTGCTGTGGATGTT	GAGAGGGAGTGTCTGCTTGG

Gene Symbol	Gene Name	Forward Primer (5'-3')	Reverse Primer (5'-3')
IL8R α	Interleukin 8 receptor, α	GGTCTGCTACGGGCTCACA	CCCGGGGTTTCTGTCTCAT
IL10	Interleukin 10	CCCTGGAGAGAGAGCTGAAGA	CACTGCCTTGTCTTTTATTTCTACA
IL10R α	Interleukin 10 receptor, α	GCGCACAGGGGACAGAAC	GCTGGAAAAAATCTGGCTTCAA
IL11	Interleukin 11	CTGGATAGCGCTGTCTCTCTTG	TGTC'TCTCAITCTGTGCAGCTAGTTG
IL12 α	Interleukin 12 α	TCTGGACCTGCCAAGTGCTTT	TGGCGGTCTCACCACTGT
IL12 β	Interleukin 12 β	AGAGGAGGAGTGTAAACAGAAAAAGGT	CGCCCTTTTGCAITGG
IL13	Interleukin 13	GCAACATCACACAAGACCAGAAG	TGTACAGTCCACGCTCCAT
IL15	Interleukin 15	CCCTCTCAACAGTCACTTCTTT	ACAGAGGCCAACTGGATA
IL16	Interleukin 16	CCAGTCCCCATCATCGTTAGC	CCAAACTTGACGCCCTTCCA
IL17 β	Interleukin 17 β	AGCTGCAGGCTGACTTAATGG	AGATGGAGATGCAAGAAAGGAA
IL18	Interleukin 18	AAACCCGCTGTGTTTGA	TCAGTCTGGTCTGGGATTCTGT
IL33	Interleukin 33	CCAAAGGAACCTTCACTGTCTAACAGA	GTAACATCCATTCCTCAAAACAAAAAC
IRF1	Interferon regulatory factor 1	CATTCCTGGGTACCCCTCTGT	TCCTTAGCCAGGGTCTCAITCG
IRF2	Interferon regulatory factor 2	AAGGAAACAACGCCCTTCAGAGT	TTCTCTGTCTTTTGGTTTCTTTCCCTTT
IRF3	Interferon regulatory factor 3	CTGGCTCAGGAACCTGCACGT	CCCAGGTCCAGCTGAGACA
IRF4	Interferon regulatory factor 4	CAGACTGCCGGCTGCATAT	CCAGGTTTGCTAACATCATAGGTATGT
IRF6	Interferon regulatory factor 6	CATAGAGATTCCAAACGGCTTCCA	CAAGCCTTAAAAATGGTGTCTTCTT
IRF7	Interferon regulatory factor 7	GCTCTGGAGAACAGGGAAGAAG	GTTTGTCAACCCAGCAITTTCCCT
IRF8	Interferon regulatory factor 8	TGGTGACTGGGTATACTGCTCTATG	TGCCCCCGTAGTAAAGTTGA
IRF9	Interferon regulatory factor 9	GGAAGGAGCTGGCACAAACTG	AGGAAGCGGATTCTTCCATGA
TGF β 1	Transforming growth factor, β 1	AGAAAGTCACCCGCGTGCTA	TGTGTGATGTCTTTGGGTTTGTCA
TLR4	Toll-like receptor 4	CCCTCATGACATCCCTTATTC	CAATTTCTCACAACTTCAGTGG
TNF	Tumor necrosis factor (TNF superfamily, member 2)	CCTCAGCCTCTTCTCATTT	GGAACCTTCTCTCCTTGT
TNFRSF1 α	Tumor necrosis factor receptor superfamily, member 1 α	TGCCCCATCCAAAGAATAATTCC	TGGGCTTGGACAGTCACTCA
TNFRSF1 β	Tumor necrosis factor receptor superfamily, member 1 β	GCCTAGCCAAACTCCACACATC	TCAGTCCAACGATCAGACCAAT

Effects of Nicotine and Viral Proteins on Immune-Related Genes in the Nucleus Accumbens of the F344 and HIV-1Tg Rats

Table 2

Gene Symbol	F344 rat (n=5-6 animals)				HIV-1Tg rat (n=5-6 animals)				Viral effect	
	Sal (Mean $\pm$ SD)	Nic (Mean $\pm$ SD)	Ratio Nic/Sal	Nic effect in F344 (P-value)	Sal (Mean $\pm$ SD)	Nic (Mean $\pm$ SD)	Ratio Nic/Sal	Nic effect in HIV-1Tg (P-value)	Ratio HIV-1Tg_Sal/ F344_Sal	P-value
CASP3	8.1 $\pm$ 0.8	11.4 $\pm$ 2.9	1.41	0.039	11.6 $\pm$ 1.8	11.2 $\pm$ 2.8	0.97	0.756	1.43	0.003
CCL3	0.085 $\pm$ 0.018	0.15 $\pm$ 0.041	1.76	0.005	0.15 $\pm$ 0.074	0.20 $\pm$ 0.11	1.33	0.443	1.76	0.054
CCL5	0.25 $\pm$ 0.044	0.21 $\pm$ 0.16	0.84	0.531	0.15 $\pm$ 0.046	0.18 $\pm$ 0.10	1.17	0.97	0.60	0.007
CCL11	0.034 $\pm$ 0.023	0.061 $\pm$ 0.027	1.81	0.127	0.0164 $\pm$ 0.0062	0.051 $\pm$ 0.027	3.08	0.023	0.48	0.149
CCR2	0.077 $\pm$ 0.027	0.054 $\pm$ 0.016	0.71	0.137	0.065 $\pm$ 0.020	0.191 $\pm$ 0.082	2.92	0.012	0.84	0.463
CX3CL1	275.1 $\pm$ 64.8	297.3 $\pm$ 90.7	1.08	0.635	359.9 $\pm$ 36.3	259.2 $\pm$ 44.1	0.72	0.003	1.31	0.029
CX3CR1	38.7 $\pm$ 6.6	32.8 $\pm$ 5.5	0.85	0.126	28.2 $\pm$ 5.3	28.1 $\pm$ 4.3	0.99	0.958	0.73	0.012
IL1 α	0.288 $\pm$ 0.055	0.185 $\pm$ 0.070	0.64	0.023	0.12 $\pm$ 0.031	0.204 $\pm$ 0.049	1.69	0.006	0.42	7.17E-05
IL2	0.29 $\pm$ 0.014	0.36 $\pm$ 0.14	1.21	0.468	0.305 $\pm$ 0.084	0.193 $\pm$ 0.0792	0.63	0.040	1.05	0.937
IL5	0.035 $\pm$ 0.006	0.076 $\pm$ 0.04	2.13	0.037	0.064 $\pm$ 0.054	0.075 $\pm$ 0.061	1.16	0.759	1.83	0.273
IL8R α	0.14 $\pm$ 0.06	0.232 $\pm$ 0.13	1.59	0.184	0.20 $\pm$ 0.049	0.10 $\pm$ 0.019	0.52	0.002	1.43	0.114
IL17 β	0.062 $\pm$ 0.046	0.069 $\pm$ 0.04	1.11	0.781	0.056 $\pm$ 0.015	0.031 $\pm$ 0.014	0.56	0.017	0.90	0.77
IRF4	1.2 $\pm$ 0.1	1.6 $\pm$ 0.3	1.33	0.026	1.9 $\pm$ 0.2	1.3 $\pm$ 0.5	0.68	0.041	1.58	0.024
IRF7	0.82 $\pm$ 0.13	1.4 $\pm$ 0.9	1.70	0.109	1.6 $\pm$ 0.03	2.1 $\pm$ 0.5	1.31	0.142	1.95	2.27E-04
IRF9	8.1 $\pm$ 0.8	11.4 $\pm$ 2.2	1.41	0.002	9.2 $\pm$ 1.4	8.8 $\pm$ 2.0	0.96	0.741	1.14	0.139
TGF β 1	3.5 $\pm$ 0.3	3.3 $\pm$ 0.3	0.94	0.517	3.0 $\pm$ 0.2	3.2 $\pm$ 0.3	1.01	0.88	0.86	0.023
TLR4	1.73 $\pm$ 0.32	1.70 $\pm$ 0.31	0.97	0.825	1.32 $\pm$ 0.2	1.3 $\pm$ 0.1	0.98	0.843	0.76	0.030
TNF	0.32 $\pm$ 0.096	0.29 $\pm$ 0.062	0.89	0.454	0.25 $\pm$ 0.1	0.13 $\pm$ 0.065	0.52	0.044	0.78	0.217

Table 3

Effects of Nicotine and Viral Proteins on Immune-Related Genes in the PFC of the F344 and HIV-1Tg Rat

Gene Symbol	F344 rat (n=5-6 animals)				HIV-1Tg rat (n=5-6 animals)				Viral effect	
	Sal (Mean $\pm$ SD)	Nic (Mean $\pm$ SD)	Ratio Nic/Sal	Nic effect in F344 (P-value)	Sal (Mean $\pm$ SD)	Nic (Mean $\pm$ SD)	Ratio Nic/Sal	Nic effect in HIV-1Tg (P-value)	Ratio HIV-1Tg_Sal/ F344_Sal	P-value
CASP1	0.25 $\pm$ 0.061	0.20 $\pm$ 0.062	0.80	0.206	0.30 $\pm$ 0.059	0.20 $\pm$ 0.026	0.66	0.006	1.20	0.191
CCL2	0.15 $\pm$ 0.040	0.080 $\pm$ 0.013	0.51	0.004	0.093 $\pm$ 0.043	0.078 $\pm$ 0.023	0.84	0.476	0.62	0.042
CCL5	0.16 $\pm$ 0.064	0.11 $\pm$ 0.015	0.689	0.131	0.078 $\pm$ 0.022	0.092 $\pm$ 0.011	1.19	0.192	0.49	0.026
CCR5	0.014 $\pm$ 0.005	0.012 $\pm$ 0.0073	0.92	0.752	0.015 $\pm$ 0.004	0.010 $\pm$ 0.0017	0.66	0.031	1.07	0.72
CX3CR1	21.7 $\pm$ 5.69	17.1 $\pm$ 2.44	0.79	0.129	19.9 $\pm$ 4.66	14.4 $\pm$ 2.80	0.72	0.035	0.92	0.548
IL1 β	1.48 $\pm$ 0.51	1.30 $\pm$ 0.42	0.88	0.512	1.59 $\pm$ 0.27	1.00 $\pm$ 0.31	0.63	0.014	1.07	0.943
IL1R2	0.13 $\pm$ 0.021	0.09 $\pm$ 0.018	0.68	0.011	0.14 $\pm$ 0.034	0.074 $\pm$ 0.028	0.53	0.004	1.08	0.615
IL8Ra	0.36 $\pm$ 0.060	0.045 $\pm$ 0.024	0.12	0.267	0.91 $\pm$ 0.81	0.027 $\pm$ 0.017	0.30	0.025	2.53	0.254
IL18	2.94 $\pm$ 0.95	1.82 $\pm$ 0.36	0.63	0.023	2.08 $\pm$ 0.85	1.51 $\pm$ 0.73	0.73	0.245	0.71	0.133
TGF β 1	2.81 $\pm$ 0.38	2.12 $\pm$ 0.29	0.76	0.006	2.17 $\pm$ 0.37	1.69 $\pm$ 0.31	0.74	0.040	0.77	0.015
TLR4	1.60 $\pm$ 0.41	1.05 $\pm$ 0.25	0.66	0.031	0.87 $\pm$ 0.28	0.93 $\pm$ 0.16	1.08	0.626	0.54	0.010
TNF	0.18 $\pm$ 0.091	0.14 $\pm$ 0.058	0.83	0.492	0.14 $\pm$ 0.053	0.077 $\pm$ 0.043	0.53	0.036	0.78	0.428
TNFRSF1 α	3.83 $\pm$ 0.47	3.15 $\pm$ 0.35	0.82	0.019	3.37 $\pm$ 0.72	2.47 $\pm$ 0.29	0.73	0.017	0.88	0.225

Effects of Nicotine and Viral Proteins on Immune-Related Genes in the VTA of the F344 and HIV-1Tg Rat

Table 4

Gene Symbol	F344 rat (n=5-6 animals)				HIV-1Tg rat (n=5-6 animals)				Viral effect	
	Sal (Mean $\pm$ SD)	Nic (Mean $\pm$ SD)	Ratio Nic/Sal	Nic effect in F344 (P-value)	Sal (Mean $\pm$ SD)	Nic (Mean $\pm$ SD)	Ratio Nic/Sal	Nic effect in HIV-1Tg (P-value)	Ratio HIV-1Tg_Sal/ F344_Sal	P-value
CASP3	6.3 $\pm$ 0.83	4.61 $\pm$ 0.85	0.73	0.008	4.60 $\pm$ 0.43	4.30 $\pm$ 1.12	0.94	0.556	1.37	0.001
CCL2	0.206 $\pm$ 0.053	0.137 $\pm$ 0.014	0.67	0.022	0.160 $\pm$ 0.054	0.114 $\pm$ 0.047	0.71	0.150	1.29	0.169
CCR5	0.054 $\pm$ 0.027	0.045 $\pm$ 0.033	0.83	0.632	0.064 $\pm$ 0.028	0.0196 $\pm$ 0.0123	0.30	0.005	0.84	0.527
CX3CR1	28.2 $\pm$ 7.8	22.2 $\pm$ 0.7	0.79	0.124	19.6 $\pm$ 4.8	16.5 $\pm$ 3.8	0.84	0.255	1.44	0.046
IFNa1	1.05 $\pm$ 0.12	1.15 $\pm$ 0.18	1.10	0.283	0.79 $\pm$ 0.17	0.7 $\pm$ 0.16	0.88	0.359	1.33	0.015
IL1 β	0.470 $\pm$ 0.12	0.42 $\pm$ 0.34	0.91	0.772	0.163 $\pm$ 0.050	0.15 $\pm$ 0.11	0.95	0.876	2.88	0.0005
IL5	0.097 $\pm$ 0.036	0.108 $\pm$ 0.053	1.11	0.718	0.21 $\pm$ 0.11	0.088 $\pm$ 0.029	0.42	0.036	0.46	0.110
IL6	0.072 $\pm$ 0.055	0.0228 $\pm$ 0.0368	0.31	0.192	0.28 $\pm$ 0.17	0.257 $\pm$ 0.289	0.91	0.874	0.26	0.033
IL8Ra	0.069 $\pm$ 0.017	0.30 $\pm$ 0.11	4.34	0.0009	0.13 $\pm$ 0.10	0.062 $\pm$ 0.033	0.45	0.118	0.53	0.137
IL33	8.07 $\pm$ 0.73	9.49 $\pm$ 0.99	1.18	0.018	8.4 $\pm$ 2.4	8.1 $\pm$ 0.7	0.96	0.79	0.96	0.757
IRF1	3.8 $\pm$ 1.6	3.7 $\pm$ 0.8	0.97	0.973	3.6 $\pm$ 1.0	2.4 $\pm$ 0.3	0.67	0.032	1.06	0.836
IRF6	0.91 $\pm$ 0.26	0.53 $\pm$ 0.18	0.58	0.020	0.97 $\pm$ 0.21	1.24 $\pm$ 0.63	1.28	0.353	0.94	0.710
TNF	0.2 $\pm$ 0.077	0.3 $\pm$ 0.1	1.50	0.274	0.2 $\pm$ 0.069	0.1 $\pm$ 0.068	0.50	0.008	1.00	0.652

Table 5

Comparison of differentially expressed number genes by HIV-1 viral proteins and nicotine in the NAs, PFC and VTA regions

Effect of HIV-1 viral proteins and/or nicotine	NAc		PFC		VTA	
	Up-regulated	Down-regulated	Up-regulated	Down-regulated	Up-regulated	Down-regulated
Nicotine	5	1	0	6	1	3
HIV-1 viral proteins	4	5	0	4	3	1
Nicotine + HIV-1 viral proteins	3	6	0	9	0	4