Published in final edited form as:
Infect Genet Evol. 2013 December ; 20: 78–82. doi:10.1016/j.meegid.2013.08.008.

Association between *TLR3* rs3775291 and resistance to HIV among highly exposed Caucasian intravenous drug users

Kristi Huik[a,*], **Radko Avi**[a], **Merit Pauskar**[a], **Eveli Kallas**[a], **Ene-Ly Jõgeda**[a], **Tõnis Karki**[a], **Kristina Marsh**[b], **Don Des Jarlais**[c], **Anneli Uusküla**[b], and **Irja Lutsar**[a]

Kristi Huik: kristi.huik@ut.ee

[a]Department of Microbiology, Faculty of Medicine, University of Tartu, Ravila 19, Tartu 50411, Estonia

[b]Department of Public Health, Faculty of Medicine, University of Tartu, Ravila 19, Tartu 50411, Estonia

[c]Edmond de Rothschild Chemical Dependency Institute, Beth Israel Medical Center, West 57th Street, New York, NY 10019, USA

Abstract

Background—TLR3 recognizes dsRNA and triggers immune responses against RNA and DNA viruses. A polymorphism in *TLR3*, rs3775291 (Leu412Phe), has been associated with the increased susceptibility to enteroviral myocarditis, protection against tick-borne encephalitis virus and HIV-1 infection. We investigated Caucasian intravenous drug users (IDUs) and blood donors in order to evaluate the associations between *TLR3* genotypes and susceptibility to HIV infection.

Materials and methods—A total of 345 Caucasian IDUs were recruited, 50% of them were HIV positive, 89% HCV and 77% HBV positive. Based on their history of needle sharing, 20 of the HIV negative IDUs were classified as highly exposed HIV seronegatives (HESNs), 68 as non-HESNs and 85 as unexposed. The control group consisting of 497 blood donors tested negative for all three viruses. *TLR3* rs3775291 were determined by using TaqMan Allelic Discrimination Assay.

Results—The *TLR3* rs3775291 T allele frequency was similar among the HIV negative and HIV positive IDUs and blood donors – 36%, 31% and 34%, respectively. The frequency of persons possessing at least one *TLR3* rs3775291 T allele was significantly higher in HESNs compared with blood donors and HIV positive IDUs (80% vs. 55%; $p = 0.037$ and 80% vs. 53%; $p = 0.031$, respectively). In the univariate analysis, persons who possessed at least one T allele had reduced odds of being HIV seropositive (OR = 0.29, 95% CI = 0.09–0.90). This association remained significant (OR = 0.25, 95% CI = 0.07–0.87) after the adjustment for other co-variates (HCV, HBV serostatus and duration of intravenous drug use).

*Corresponding author. Tel.: +372 7374185.

Appendix A. Supplementary data: Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.meegid.2013.08.008.

Conclusions—The *TLR3* rs3775291 T allele has a protective effect against HIV infection among HESNs IDUs.

Keywords

TLR3; Leu412Phe; Intravenous drug users; Highly exposed HIV seronegatives

1. Introduction

During the last decade a number of different host genetic factors [e.g. single nucleotide polymorphisms (SNPs), gene copy number] have been found to play a role in human immunodeficiency virus (HIV) acquisition. In addition to SNPs in HIV targeted genes such as CCR5, CCL5 and CCL3L1 (Gonzalez et al., 1999, 2001, 2005; Huik et al., 2010) the toll-like receptor 3 (TLR3) has been demonstrated to have influence on susceptibility of HIV infection (Sironi et al., 2012).

TLRs are pattern-recognition receptors that activate innate immune response in humans and thus play a key role in immune response (Lee et al., 2012). TLR3 is one of the four TLRs (TLR3, TLR7, TLR8 and TLR9) that recognize double-stranded RNA and trigger immune responses against both RNA and DNA viruses by stimulating type I interferons (INFs) and inflammatory cytokines (Alexopoulou et al., 2001; Matsumoto et al., 2004). TLR3 is expressed within the endosomal compartment of conventional dendritic cells (Muzio et al., 2000; Visintin et al., 2001), macrophages (Jiang et al., 2005), T lymphocytes (Tabiasco et al., 2006), fibroblasts (Rudd et al., 2005) and hepatocytes (Lang et al., 2006). In addition, TLR3 is expressed in epithelial cells as well as on their surface (Sha et al., 2004; Uehara et al., 2007).

TLR3 ligand-binding leads to the activation of the transcription factors like interferon-regulatory factor-3 and nuclear factor-κB (NF-kB) followed by induction of INF-β and proinflammatory cytokines (Matsumoto et al., 2004). The induction of INF-β activates several other genes, which contribute to the antiviral effect via the inhibition of protein synthesis or viral replication (Vercammen et al., 2008). A co-stimulatory role of TLR3 on human T lymphocytes has also been suggested (Tabiasco et al., 2006; Wesch et al., 2006).

A *TLR3* polymorphism rs3775291 C→T (Leu412Phe) was associated with the increased risk of enteroviral myocarditis (Gorbea et al., 2010) and of herpes simplex-1 encephalitis (Zhang et al., 2007) but protects against tick-borne encephalitis (Kindberg et al., 2011). Recently, a minor allele of *TLR3* rs3775291 was shown to provide resistance to HIV-1 infection in a Spanish (primary risk for injecting drug use transmission) and Italian cohorts (primary risk for sexual transmission) of highly exposed HIV seronegatives (HESNs); T homozygosity was overrepresented among the HESNs as compared to healthy volunteers (Sironi et al., 2012).

The Estonian HIV epidemic among IDUs is characterized by homogeneity – the population consists predominantly of young male subjects of Caucasian origin of whom the vast majority is infected with HIV-1 recombinant viruses CRF06_cpx or CRF06A (Avi et al., 2010, 2011). The presence of a homogenous population provides an excellent opportunity to

explore the associations between host genetic factors and HIV acquisition without the complexities that would occur if many different viral strains or ethnic groups were present.

In this study we aimed to evaluate the role of *TLR3* genotypes on the susceptibility of HIV, HCV and HBV infection in Caucasian IDUs.

2. Materials and methods

2.1. Subjects and study groups forming

A total of 345 Caucasian IDUs were recruited by using a respondent-driven sampling methodology (Malekinejad et al., 2008) in the syringe-exchange program in the capital of Estonia, Tallinn in 2011. All participants underwent face-to-face interviews using a structured questionnaire administered by the specially trained staff to collect information on demographics and risk behavior (the duration of intravenous drug use (IVDU), the history of sharing syringes).

Overall 172 (50%) IDUs were HIV positive. Of the 173 HIV negative IDUs 88 reported using previously used syringes (receptive sharing) and 85 did not admit to the repetitive usage. They were called unexposed and were excluded from further analysis. The receptive sharing group was further stratified based on the extent of exposure during the last 6 months flowingly: persons who had shared syringes at least once a month were considered highly exposed seronegatives (HESNs; $n = 20$) and those sharing syringes less frequently were called non-HESNs ($n = 68$).

Similar groups were derived for HCV and HBV negative subjects. First, persons who were HCV or HBV negative and reported the use of previously used syringes less than once a month were defined as HCV or HBV negative non-highly exposed IDUs, respectively. Second, persons who were HCV or HBV negative and reported the use of previously used syringes at least once a month were defined as HCV or HBV negative highly exposed IDUs, respectively.

2.2. Sample collection

Two 8 ml samples of venous blood were collected via venepuncture into EDTA tubes. The first sample was used for the detection of anti-HIV-1 and the second was used for the separation of cells by Ficoll gradient within 24 h. The separated plasma from the second sample was further used for the determination of HCV and HBV status as described below.

The scavenged plasma samples of 497 HIV, HCV and HBV negative blood donors of Caucasian origin were obtained from the blood donation centers in Tallinn in 2010 and were used as a control group. No other data in blood donors were available. The samples were collected into EDTA tubes, they were stored at +4 °C and after confirming their negativity of all three infections they were sent to our laboratory.

All plasma samples and peripheral blood mononuclear cells or whole blood were stored at −80 °C until further analysis.

2.3. Laboratory analyses and genotyping

The detection of HIV was performed in the Estonian Central HIV Reference Laboratory and HCV, HBV testing in the Estonian Institute for Health Development. The presence of HCV and HBV antibodies was detected with the ETI-AB-HCVK-3 anti-HCV test (DiaSorin, Vercelli, Italy) and ETI-MAK-4 HBsAg (DiaSorin, Vercelli, Italy), ETIAB-COREK Plus (anti-HBc, DiaSorin, Vercelli, Italy), respectively. HBV positivity was defined as positivity for anti-HBc or HBsAg. The detection of HBs antibody (HBsAb, DiaSorin, Vercelli, Italy) was used to determine the rate of vaccination against HBV infection. HIV testing was performed by using a fourth generation enzyme-linked immunoassay (Abbott IMx HIV-1/HIV-2 III Plus, Abbott Laboratories, Abbott Park, IL, USA) and was confirmed by the immunoblotting (INNO LIA HIV I/II Score Western blot (Microgen Bioproducts Ltd., Surrey, UK).

For the detection of *TLR3* rs3775291 genotypes human genomic DNA was extracted from the whole blood or PBMCs using the Pure-Link® *Pro* 96 Genomic DNA Purification Kit (Invitrogen, Carlsbad, CA, USA). The *TLR3* rs3775291 was detected by TaqMan Allelic Discrimination Assay (cat No. 4351379, Applied Biosystems, Foster City, CA, USA).

2.4. Statistical analyses

Statistical analyses were performed using R 2.13.1 program (last accessed July 1st, 2012) and a two-tailed p value of <0.05 was considered statistically significant. The differences in the distribution of *TLR3* rs3775291 were compared by Fisher exact test and the p-values were not corrected for multiple comparison. The associations between *TLR3* rs3775291 and HIV seropositivity were evaluated by uni- and multivariate logistic regression.

2.5. Ethical considerations

The Ethics Committee of the University of Tartu approved this study. All IDUs gave an informed consent for the participation of the study and blood donors agreed with using leftover blood for research purposes. Blood donation is unpaid in Estonia.

3. Results

3.1. Characteristics and co-infection status of the study groups

The characteristics of IDUs population are presented in Table 1. In total, half of IDUs were infected with HIV, 89% with HCV, 77% with HBV and 40% had HIV/HCV/HBV triple infection. There were no significant differences in the distribution of age, gender and the duration of IVDU among the subgroups but HIV positive IDUs were more likely to be HBV and/or HCV seropositive compared to non-HESNs, HESNs and unexposed IDUs (all $p < 0.05$).

3.2. Distribution of TLR3 rs3775291 among study groups and associations with HIV seropositivity

All samples of IDUs and blood donors were successfully genotyped for *TLR3* rs3775291 polymorphism. The T allele frequency was 36% among HIV negative IDUs (48% in

HESNs, 34% in non-HESNs and 35% in unexposed IDUs), 31% among HIV positive IDUs and 34% among blood donors, and was in Hardy–Weinberg equilibrium in all the groups.

We did not find any significant differences in the prevalence of *TLR3* rs3775291 genotypes and no possession of at least one T allele when comparing the exposed HIV negative IDUs (HESNs and non-HESNs together) with blood donors or with HIV positive IDUs (data not shown).

In order to evaluate the effect of *TLR3* rs3775291 genotypes we compared non-HESNs, HESNs with blood donors and HIV positive IDUs. The frequencies of *TLR3* rs3775291 genotypes in different study groups are presented in Table 2. The distribution of *TLR3* rs3775291 genotypes did not differ among the non-HESNs, HESNs and blood donors (Supplementary Fig. 1, left panel). However, the number of persons who possessed at least one T allele was significantly greater among HESNs than in blood donors (80% vs. 55%; $p = 0.037$) and HIV positive IDUs (80% vs. 53%; $p = 0.031$). The possession of at least one T allele did not differ between non-HESNs and other groups (Supplementary Fig. 1, right panel).

The possession of *TLR3* T allele in the univariate analysis was associated with the decreased odds of HIV infection, while the HCV and HBV seropositivity as well as the each-year intravenous drug use increased the odds of being HIV positive (Table 3). The possession of *TLR3* T allele and HCV seropositive status remained significantly associated with being HIV seropositive in multivariate analysis (Table 3).

Next, we compared the distribution of *TLR3* rs3775291 T allele in highly exposed HIV positive IDUs (using the same receptive sharing criteria as in the case of HIV negative IDUs) and HESNs. The T allele frequency was lower among the highly exposed HIV positive IDUs than in the HESNs but did not reach to statistical significance (54% vs. 80%, respectively; $p = 0.055$).

Since TLR3 has the ability to recognize HBV and HCV, we examined the distributions of *TLR3* rs3775291 T allele among the different exposure groups for HBV and HCV. There were no statistically significant differences in distribution of *TLR3* rs3775291 T allele between these groups (Supplementary Table 1).

4. Discussion

The use of intravenous drugs is one of the most important factors in the acquisition of blood-borne infections including HIV (Mathers et al., 2008; Nelson et al., 2011). However, some persons who frequently share injection equipment remain HIV negative. In concordance with findings of Sironi et al. (2012) we observed that *TLR3* rs3775291 T allele was overrepresented in HESNs compared to HIV positive IDUs and blood donors, suggesting a protective effect of this T allele against acquisition of HIV infection in highly exposed subjects. However, in contrast to findings of Sironi et al. (2012), we did not observe a difference in *TLR3* rs3775291 genotype distribution. Here the differences in the subject populations and in the definitions of HESNs or in other factors might explain the discrepancies in the results.

The mechanism of how *TLR3* rs3775291 affects the susceptibility/resistance to infections is not entirely clear. In the enteroviral infection Gorbea et al. (2010) demonstrated the *TLR3* rs3775291 T allele influence on TLR3 signaling leading to the increased risk of infection. They showed that in the cell culture T homozygotes had reduced NF-kB and type I interferon signaling after stimulation with the synthetic ligand for TLR3–poly(I:C). After infecting culture with Coxsackie virus B3 (the virus most commonly associated with viral cardiomyopathies) type I interferon signaling was significantly reduced which resulted in increased viral replication. This suggests to a functional impairment of the molecule in terms of mediating signaling (Gorbea et al., 2010; Ranjith-Kumar et al., 2007). In the case of flaviviral encephalitis on the other hand it has been proposed that the impaired TLR3 signal produces a milder immune response and this leads to an improved outcome (Kindberg et al., 2011). Together, these results suggest the effect of TLR3 might depend on the type of virus involved.

The debate whether TLR3 recognizes HIV or not is still ongoing. It has been suggested that after retroviral genome dimerizes and forms a secondary structure it is likely that a double-stranded RNA is formed (Russell et al., 2004; Greatorex, 2004; Watts et al., 2009). The fact that there may be interactions between retroviruses and TLR3 is demonstrated by Miyauchi et al. (2012). Using a xenotropic murine leukemia virus-related virus as a model they showed that TLR3 is able to recognize retroviral genome and thus evoke an antiviral response.

How *TLR3* polymorphisms protect against HIV is not entirely clear. Sironi et al. (2012) have excluded the possibility of the association between TLR3 expression on CD4+ and CD14+ cells and responsiveness to stimulation by HIV (Sironi et al., 2012). Nevertheless, they demonstrated that *TLR3* rs3775291 heterozygotes have higher cytokine (CCL3 and IL-6) mRNA expression compared to C homozygotes suggesting that higher production of cytokines may protect against HIV. In addition, CCL3 is a ligand for the HIV co-receptor CCR5 that has anti-R5 activity (Abdelwahab et al., 2003). Thus, the overexpression of CCL3 may result in the competition for the receptor occupancy and the prevention of HIV entry. Furthermore, heterozygotes have higher expression of CD69 (a marker for early activated T lymphocytes) than C homozygotes (Sironi et al., 2012) which may indicate a more active immune response.

The association with HIV infection we observed may not be the direct effect of the rs3775291 T allele, but might relate to another SNP that is in linkage disequilibrium with the rs3775291 T allele, (e.g. rs10025405, SNPs in position −7 and IVS3 +71) (Noguchi et al., 2004). However, the rs3775291 T is the only non-synonymous mutation in TLR3 at appreciable frequency in European populations. This would suggest that rs3775291 is the most likely SNP influencing susceptibility to HIV infection in a European population like in Estonia. There is also a possibility that SNPs in linkage disequilibrium with rs3775291 could contribute to the regulatory processes. Determining the exact mechanism behind the association between *TLR3* rs3775291 and HIV susceptibility will require further research.

The main limitation of the study is a small proportion of HESNs. This group of subjects is a huge challenge to recruit as most highly exposed IDUs do become infected with HIV. That

we did observe a statistically significant association between *TLR3* rs3775291 T allele among HESNs compared to HIV seropositive subjects in both the univariate and the multivariate logistic regressions suggests a strong association. A second limitation is the categorization of risk behavior patterns according to the self-report. The questionnaire was administered by the trained study personnel. Direct observation of injecting risk behavior is not practical, so that studies such as this one will necessarily have to rely on self-report data. Misclassification of the extent of injecting risk behavior would be more likely to bias the results towards null findings rather than create false positive findings. Regardless of these limitations we believe that the data presented here reliably describe the role of *TLR3* T-allele in acquisition of HIV infection.

In conclusion, our study conducted among Caucasian IDUs confirmed findings of Sironi et al. on a protective association of the *TLR3* rs3775291 T allele against HIV infection, suggesting the immunologically mediated protection from HIV infection through TLR3. The mechanism through which such a protective effect might occur remains to be determined.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank the study participants and teams from the nongovernmental organization “Convictus”.

The work was supported by European Union through the European Regional Development Fund; Estonian Science Foundation (Grants 8415 and 8856); Basic Financing and the Target Financing of Estonian Ministry of Education and Research (SF0180004s12); and Grant R01 DA003574 from the US National Institutes of Health.

References

Abdelwahab SF, Cocchi F, Bagley KC, Kamin-Lewis R, Gallo RC, DeVico A, Lewis GK. HIV-1-suppressive factors are secreted by CD4+ T cells during primary immune responses. Proc Natl Acad Sci USA. 2003; 100:15006–15010. [PubMed: 14657379]

Alexopoulou L, Holt AC, Medzhitov R, Flavell RA. Recognition of double-stranded RNA and activation of NF-kappaB by Toll-like receptor 3. Nature. 2001; 413:732–738. [PubMed: 11607032]

Avi R, Huik K, Pauskar M, Ustina V, Karki T, Krispin T, Ainsalu K, Paap P, Schmidt J, Nikitina N, Lutsar I. Emerging transmitted drug resistance in treatment-naive human immunodeficiency virus-1 CRF06_cpx-infected patients in Estonia. Scand J Infect Dis. 2011; 43:122–128. [PubMed: 20964489]

Avi R, Huik K, Sadam M, Karki T, Krispin T, Ainsalu K, Paap P, Schmidt J, Nikitina N, Lutsar I. Characterization of integrase region polymorphisms in HIV type 1 CRF06_cpx viruses in treatment-naive patients in Estonia. AIDS Res Hum Retroviruses. 2010; 26:1109–1113. [PubMed: 20849300]

Gonzalez E, Bamshad M, Sato N, Mummidi S, Dhanda R, Catano G, Cabrera S, McBride M, Cao XH, Merrill G, O'Connell P, Bowden DW, Freedman BI, Anderson SA, Walter EA, Evans JS, Stephan KT, Clark RA, Tyagi S, Ahuja SS, Dolan MJ, Ahuja SK. Race-specific HIV-1 disease-modifying effects associated with CCR5 haplotypes. Proc Natl Acad Sci USA. 1999; 96:12004–12009. [PubMed: 10518566]

Gonzalez E, Dhanda R, Bamshad M, Mummidi S, Geevarghese R, Catano G, Anderson SA, Walter EA, Stephan KT, Hammer MF, Mangano A, Sen L, Clark RA, Ahuja SS, Dolan MJ, Ahuja SK. Global survey of genetic variation in CCR5, RANTES, and MIP-1alpha: impact on the epidemiology of the HIV-1 pandemic. Proc Natl Acad Sci USA. 2001; 98:5199–5204. [PubMed: 11320252]

Gonzalez E, Kulkarni H, Bolivar H, Mangano A, Sanchez R, Catano G, Nibbs RJ, Freedman BI, Quinones MP, Bamshad MJ, Murthy KK, Rovin BH, Bradley W, Clark RA, Anderson SA, O'Connell RJ, Agan BK, Ahuja SS, Bologna R, Sen L, Dolan MJ, Ahuja SK. The influence of CCL3L1 gene-containing segmental duplications on HIV-1/AIDS susceptibility. Science. 2005; 307:1434–1440. [PubMed: 15637236]

Gorbea C, Makar KA, Pauschinger M, Pratt G, Bersola JL, Varela J, David RM, Banks L, Huang CH, Li H, Schultheiss HP, Towbin JA, Vallejo JG, Bowles NE. A role for Toll-like receptor 3 variants in host susceptibility to enteroviral myocarditis and dilated cardiomyopathy. J Biol Chem. 2010; 285:23208–23223. [PubMed: 20472559]

Greatorex J. The retroviral RNA dimer linkage: different structures may reflect different roles. Retrovirology. 2004; 1:22. [PubMed: 15317659]

Huik K, Sadam M, Karki T, Avi R, Krispin T, Paap P, Ruutel K, Uuskula A, Talu A, Abel-Ollo K, Lutsar I. CCL3L1 copy number is a strong genetic determinant of HIV seropositivity in Caucasian intravenous drug users. J Infect Dis. 2010; 201:730–739. [PubMed: 20095832]

Jiang W, Sun R, Wei H, Tian Z. Toll-like receptor 3 ligand attenuates LPS-induced liver injury by down-regulation of Toll-like receptor 4 expression on macrophages. Proc Natl Acad Sci USA. 2005; 102:17077–17082. [PubMed: 16287979]

Kindberg E, Vene S, Mickiene A, Lundkvist A, Lindquist L, Svensson L. A functional Toll-like receptor 3 gene (TLR3) may be a risk factor for tick-borne encephalitis virus (TBEV) infection. J Infect Dis. 2011; 203:523–528. [PubMed: 21216866]

Lang KS, Georgiev P, Recher M, Navarini AA, Bergthaler A, Heikenwalder M, Harris NL, Junt T, Odermatt B, Clavien PA, Pircher H, Akira S, Hengartner H, Zinkernagel RM. Immunoprivileged status of the liver is controlled by Toll-like receptor 3 signaling. J Clin Invest. 2006; 116:2456–2463. [PubMed: 16955143]

Lee CC, Avalos AM, Ploegh HL. Accessory molecules for Toll-like receptors and their function. Nat Rev Immunol. 2012; 12:168–179. [PubMed: 22301850]

Malekinejad M, Johnston LG, Kendall C, Kerr LR, Rifkin MR, Rutherford GW. Using respondent-driven sampling methodology for HIV biological and behavioral surveillance in international settings: a systematic review. AIDS Behav. 2008; 12:S105–130. [PubMed: 18561018]

Mathers BM, Degenhardt L, Phillips B, Wiessing L, Hickman M, Strathdee SA, Wodak A, Panda S, Tyndall M, Toufik A, Mattick RP. Global epidemiology of injecting drug use and HIV among people who inject drugs: a systematic review. Lancet. 2008; 372:1733–1745. [PubMed: 18817968]

Matsumoto M, Funami K, Oshiumi H, Seya T. Toll-like receptor 3: a link between toll-like receptor, interferon and viruses. Microbiol Immunol. 2004; 48:147–154. [PubMed: 15031527]

Miyauchi K, Urano E, Takeda S, Murakami T, Okada Y, Cheng K, Yin H, Kubo M, Komano J. Toll-like receptor (TLR) 3 as a surrogate sensor of retroviral infection in human cells. Biochem Biophys Res Commun. 2012; 424:519–523. [PubMed: 22771581]

Muzio M, Bosisio D, Polentarutti N, D'Amico G, Stoppacciaro A, Mancinelli R, van't Veer C, Penton-Rol G, Ruco LP, Allavena P, Mantovani A. Differential expression and regulation of toll-like receptors (TLR) in human leukocytes: selective expression of TLR3 in dendritic cells. J Immunol. 2000; 164:5998–6004. [PubMed: 10820283]

Nelson PK, Mathers BM, Cowie B, Hagan H, Des Jarlais D, Horyniak D, Degenhardt L. Global epidemiology of hepatitis B and hepatitis C in people who inject drugs: results of systematic reviews. Lancet. 2011; 378:571–583. [PubMed: 21802134]

Noguchi E, Nishimura F, Fukai H, Kim J, Ichikawa K, Shibasaki M, Arinami T. An association study of asthma and total serum immunoglobin E levels for Toll-like receptor polymorphisms in a Japanese population. Clin Exp Allergy. 2004; 34:177–183. [PubMed: 14987294]

Ranjith-Kumar CT, Miller W, Sun J, Xiong J, Santos J, Yarbrough I, Lamb RJ, Mills J, Duffy KE, Hoose S, Cunningham M, Holzenburg A, Mbow ML, Sarisky RT, Kao CC. Effects of single nucleotide polymorphisms on Tolllike receptor 3 activity and expression in cultured cells. J Biol Chem. 2007; 282:17696–17705. [PubMed: 17434873]

Rudd BD, Burstein E, Duckett CS, Li X, Lukacs NW. Differential role for TLR3 in respiratory syncytial virus-induced chemokine expression. J Virol. 2005; 79:3350–3357. [PubMed: 15731229]

Russell RS, Liang C, Wainberg MA. Is HIV-1 RNA dimerization a prerequisite for packaging? yes, no, probably? Retrovirology. 2004; 1:23. [PubMed: 15345057]

Sha Q, Truong-Tran AQ, Plitt JR, Beck LA, Schleimer RP. Activation of airway epithelial cells by toll-like receptor agonists. Am J Respir Cell Mol Biol. 2004; 31:358–364. [PubMed: 15191912]

Sironi M, Biasin M, Cagliani R, Forni D, De Luca M, Saulle I, Lo Caputo S, Mazzotta F, Macias J, Pineda JA, Caruz A, Clerici M. A common polymorphism in TLR3 confers natural resistance to HIV-1 infection. J Immunol. 2012; 188:818–823. [PubMed: 22174453]

Zhang SY, Jouanguy E, Ugolini S, Smahi A, Elain G, Romero P, Segal D, Sancho-Shimizu V, Lorenzo L, Puel A, Picard C, Chapgier A, Plancoulaine S, Titeux M, Cognet C, von Bernuth H, Ku CL, Casrouge A, Zhang XX, Barreiro L, Leonard J, Hamilton C, Lebon P, Heron B, Vallee L, Quintana-Murci L, Hovnanian A, Rozenberg F, Vivier E, Geissmann F, Tardieu M, Abel L, Casanova JL. TLR3 deficiency in patients with herpes simplex encephalitis. Science. 2007; 317:1522–1527. [PubMed: 17872438]

Tabiasco J, Devevre E, Rufer N, Salaun B, Cerottini JC, Speiser D, Romero P. Human effector CD8+ T lymphocytes express TLR3 as a functional coreceptor. J Immunol. 2006; 177:8708–8713. [PubMed: 17142772]

Uehara A, Fujimoto Y, Fukase K, Takada H. Various human epithelial cells express functional Toll-like receptors, NOD1 and NOD2 to produce antimicrobial peptides, but not proinflammatory cytokines. Mol Immunol. 2007; 44:3100–3111. [PubMed: 17403538]

Watts JM, Dang KK, Gorelick RJ, Leonard CW, Bess JW Jr, Swanstrom R, Burch CL, Weeks KM. Architecture and secondary structure of an entire HIV-1 RNA genome. Nature. 2009; 460:711–716. [PubMed: 19661910]

Vercammen E, Staal J, Van Den Broeke A, Haegman M, Vereecke L, Schotte P, Beyaert R. Prolonged exposure to IL-1beta and IFNgamma induces necrosis of L929 tumor cells via a p38MAPK/NF-kappaB/NO-dependent mechanism. Oncogene. 2008; 27:3780–3788. [PubMed: 18246123]

Wesch D, Beetz S, Oberg HH, Marget M, Krengel K, Kabelitz D. Direct costimulatory effect of TLR3 ligand poly(I:C) on human gamma delta T lymphocytes. J Immunol. 2006; 176:1348–1354. [PubMed: 16424161]

Visintin A, Mazzoni A, Spitzer JH, Wyllie DH, Dower SK, Segal DM. Regulation of Toll-like receptors in human monocytes and dendritic cells. J Immunol. 2001; 166:249–255. [PubMed: 11123299]

Table 1

The characteristics of HIV positive, non-heavily exposed HIV seronegative subjects (non-HESNs), HESNs and unexposed IDUs.

Co-variates	**HIV infected IDUs *N* = 172**	**HIV uninfected IDUs *N* = 173**		
		Non-HESNs *N* = 68	**HESNs *N* = 20**	**Unexposed *N* = 85**
Gender				
Male (*n*; %)	133; 77	58; 85	15; 75	66; 78
Age				
Years [median (IQR)]	30 (27–33)	32 (24–37)	28 (24–33)	29 (24–33)
Duration of IVDU				
Years [median (IQR)]	12.0 (10.0–15.0)	12.0 (7.0–14.0)	9.5 (4.5–14.5)	9.0 (4.8–12.3)
Receptive syringe sharing[a]				
Yes	133; 77%	68; 100%	20; 100%	0
Receptive syringes sharing during last six months[b]				
Never (*n*; %)	96; 56	41; 60	0	85; 100
<Once a month (*n*; %)	35; 20	27; 40	0	0
≥Once a month (*n*; %)[b]	41; 24	0	20; 100	0
HCV serostatus				
HCV+ (*n*; %)	169; 98	59; 87	16; 80	62; 73
HBV serostatus[c]				
HBV+ (*n*; %)	141; 89	43; 72	9; 56	39; 57
Dual infection				
HCV+/HBV+ (*n*; %)	140; 81	42; 62	8; 40	35; 41

Note: IQR – interquartile range.

[a] Syringe sharing is categorized as persons who had ever injected with syringes that had been used by somebody else (receptive sharing).

[b] Comprises the frequencies: 1–3 times a month (*n* = 14), once a week (*n* = 2), 2–3 times a week (*n* = 2), 4–6 times a week (*n* = 1) and once a day (*n* = 1).

[c] Of HIV positive IDUs, non-HESNs, HESNs and unexposed 13, 9, 4 and 17 subjects, respectively, were vaccinated.

Table 2

The frequency of TLR3 rs3775291 genotypes in HIV positive, non-heavily exposed HIV seronegative subjects (non-HESNs), HESNs, unexposed IDUs and blood donors.

TLR3 rs3775291	HIV infected IDUs *N* = 172	HIV uninfected IDUs *N* = 173			Blood donors
		Non-HESNs *N* = 68	HESNs *N* = 20	Unexposed *N* = 85	*N* = 497
CC (*n*; %)	80; 47	31; 46	4; 20	36; 42	223; 45
CT (*n*; %)	76; 44	28; 41	13; 65	38; 45	208; 42
TT (*n*; %)	16; 9	9; 13	3; 15	11; 13	66; 13

Table 3

Uni- and multivariate analyses between HIV status and co-variates among HIV positive IDUs and HESNs.

Co-variate	Outcome: HIV positivity OR (95% CI; *p*-value)
Univariate analyses	
TLR3 rs3775291	
Non-T allele[a]	1.0
T allele	0.29 (0.09–0.90, 0.03)
Age	
Years[b]	1.03 (0.94–1.13, 0.51)
Gender	
Male[a]	1.0
Female	0.88 (0.30–2.57; 0.81)
HCV serostatus	
HCV−[a]	1.0
HCV+	14.08 (2.89–68.53; <0.01)
HBV serostatus	
HBV−[a]	1.0
HBV+	6.09 (2.02–18.36; <0.01)
Duration of intravenous drug use Years[b]	1.12 (1.01–1.24; 0.02)
Multivariate analyses	
TLR3 rs3775291	
Non-T allele[a]	1.0
T allele	0.25 (0.07–0.87; 0.03)
HCV serostatus	
HCV−[a]	1.0
HCV+	15.35 (2.46–95.69; <0.01)
HBV serostatus	
HBV−[a]	1.0
HBV+	1.25 (0.50–3.11; 0.63)
Duration of intravenous drug use Years[b]	1.11 (1.00–1.23; 0.06)

Note: OR, odds ratio; CI, confidence interval.

[a]Reference group.

[b]Years were categorized as a continuous variable in full-years, and data reflects an increase in OR with each additional year of age or intravenous drug use.