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

Kristi Huik, Radko Avi, Merit Pauskar, Eveli Kallas, Ene-Ly Jõgeda, Tõnis Karki, Kristina Marsh, Don Des Jarlais, Anneli Uusiküla, Irja Lutsar

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 (Alexopoulos 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-κB) 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 (Corbea et al., 2010) and of herpes simplex-1 encephalitis (Zhang et al., 2006). During the last decade TLR3 has been linked to 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 was 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).

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

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Ethnic groups were present. The complexities that would occur if many different viral strains or between host genetic factors and HIV acquisition without 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 scavenge 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 IDs 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...
The possession of TLR3 rs3775291 genotypes was 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 entero viral 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 Cox sackie virus B3 (the virus most commonly associated with viral cardiomyopathies) type I interferon signaling was significa-

### Table 1

<table>
<thead>
<tr>
<th>Co-variates</th>
<th>HIV infected IDUs N = 172</th>
<th>HIV uninfected IDUs N = 173</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-HESNs N = 68</td>
<td>HESNs N = 20</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male (n; %)</td>
<td>133; 77</td>
<td>58; 85</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Years [median (IQR)]</td>
<td>30 (27–33)</td>
<td>32 (24–37)</td>
</tr>
<tr>
<td>Duration of IVDU</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Years [median (IQR)]</td>
<td>12.0 (10.0–15.0)</td>
<td>12.0 (7.0–14.0)</td>
</tr>
<tr>
<td>Receptive syringe sharing(a)</td>
<td>Yes</td>
<td>133; 77</td>
</tr>
<tr>
<td>Never (n; %)</td>
<td>96; 56</td>
<td>41; 60</td>
</tr>
<tr>
<td>&lt;Once a month (n; %)</td>
<td>35; 20</td>
<td>27; 40</td>
</tr>
<tr>
<td>&gt;Once a month (n; %)(b)</td>
<td>41; 24</td>
<td>0</td>
</tr>
<tr>
<td>HCV serostatus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCV+ (n; %)</td>
<td>169; 98</td>
<td>59; 87</td>
</tr>
<tr>
<td>HBV serostatus(c)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HBV+ (n; %)</td>
<td>141; 89</td>
<td>43; 72</td>
</tr>
<tr>
<td>Dual infection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCV+/HBV+ (n; %)</td>
<td>140; 81</td>
<td>42; 62</td>
</tr>
</tbody>
</table>

**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 (13), once a week (2), 2–3 times a week (2), 4–6 times a week (1) and once a day (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.

<table>
<thead>
<tr>
<th>TLR3 rs3775291</th>
<th>HIV infected IDUs N = 172</th>
<th>HIV uninfected IDUs N = 173</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-HESNs N = 68</td>
<td>HESNs N = 20</td>
</tr>
<tr>
<td>CC (n; %)</td>
<td>80; 47</td>
<td>31; 46</td>
</tr>
<tr>
<td>CT (n; %)</td>
<td>76; 44</td>
<td>28; 41</td>
</tr>
<tr>
<td>TT (n; %)</td>
<td>16; 9</td>
<td>9; 13</td>
</tr>
</tbody>
</table>
Furthermore, heterozygotes have higher expression of CD69 (a 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. Nevertheless, they demonstrated that the data presented here reliably describe the role of TLR3 T-allele in acquisition of HIV infection.

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.

Acknowledgements

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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.

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Watts, J.M., Dang, K.K., Gorelick, R.J., Leonard, C.W., Bess Jr., J.W., Swanstrom, R., Russell, R.S., Liang, C., 2008. Prolonged exposure to IL-1β and IFN-γ induces a necrotic cell death phenotype that is partially resistant to interferon-α or TNF-α. J. Virol. 82, 7470–7483.


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