A Functional Toll-Like Receptor 8 Variant Is Associated with HIV Disease Restriction


Background. Toll-like receptors (TLRs) play an important role in the innate immune response to pathogens. TLR8 has been found to recognize RNA derived from various viruses, including human immunodeficiency virus (HIV). Presently, very little is known about the influence of TLR8 genetic variation on susceptibility to and progression of HIV disease.

Methods and results. We genotyped a population of 782 HIV-positive adults and 550 healthy control subjects for 3 nonsynonymous TLR8 single-nucleotide polymorphisms. We found that the presence of the most frequent TLR8 polymorphism, TLR8 A1G (rs3764880), confers a significantly protective effect regarding progression of the disease. In overexpression assays, we demonstrated that this receptor variant displays impaired NF-κB activation in vitro. Furthermore, we analyzed different cell types obtained from individuals differing in their TLR8 genotype and assessed their response to TLR8 ligands in vitro. The presence of the mutated receptor variant was associated with modulation of cytokine secretion profiles and lipid mediator synthesis patterns in monocytes and neutrophils.

Conclusions. This first report of a functional TLR8 variant associated with a different clinical course of an RNA viral disease may have implications for the individual risk assessment of patients infected with HIV and other RNA viruses as well as for future HIV vaccine development.
phisms (SNPs) are scarce [13]. We were able to show recently that the course of hepatitis C virus infection correlates with the presence of certain TLR7 genotypes [14]. We hypothesized that genetic variations of TLR8 might be functionally relevant and influence the progression of HIV disease. Here we show that a common TLR8 variant, which modulates cytokine secretion profiles and lipid mediator synthesis patterns, restricts HIV disease progression. Our data represent the first functional study of TLR8 genetic variation and its effect on the incidence and progression of an RNA viral disease, HIV-1 infection.

METHODS

Study population. The study group consisted of 1332 individuals, 782 HIV−1-positive adults (white, 712 [91%]; African, 26 [3%]; other, 44 [6%]; male, 711 [91%]; female 71 [9%]; median age, 39 years [interquartile range {IQR}, 34–45 years]) and 550 seronegative control subjects (white, 515 [94%]; African, 35 [6%]; male, 494 [90%]; female, 56 [10%]; median age, 39 years [IQR, 29–44 years]). Seropositive individuals were HIV-infected patients enrolled in either the German HIV-1 Seroconverter Study [15] (n = 684) or the Berlin Trial on HIV and TLR SNPs (n = 98). The German HIV-1 Seroconverter Study is a nationwide study based on a cohort of HIV-infected individuals for whom the date of seroconversion (and thus the duration of infection) can be reliably estimated, either as the midpoint between the first negative and the first positive HIV-1 antibody test result within a maximum interval of 3 years or as the date of the first reactive test result if acute seroconversion (as confirmed by laboratory diagnostic analysis) was documented. The Berlin Trial on HIV and TLR SNPs was established for the purpose of the present study and enrolled not only seroconverters (n = 24) but also seroprevalent patients (n = 74), for whom the date of seroconversion was not documented. Further details on both study cohorts are provided in appendix A, which appears only in the electronic edition of the Journal. For disease progression experiments, strictly those CD4+ T cell and virical load values obtained before the start of any antiretroviral treatment were considered, to preclude confounding effects. Disease outcome was defined by monthly CD4+ T cell count decline (data were available for 236 individuals, including 13 seroprevalent patients) or, in the analysis of 487 seroconverters, by the time from seroconversion to a CD4+ T cell count <200 cells/μL. The HIV-negative control cohort consisted of 429 anonymized seronegative white blood donors and 121 healthy, unrelated volunteers.

Informed consent was obtained from all individuals, and all investigations were conducted according to the principles expressed in the Helsinki Declaration. Written approval was obtained from the Charité-Universitätsmedizin Berlin Ethical Board and the Berlin Medical Association (Berliner Ärztekammer) Ethics Committee for all studied subjects.

Sample genotyping. A screen for new polymorphisms within the TIR domain of TLR8 was performed by polymerase chain reaction (PCR) amplification and sequence analysis for 78 individuals and revealed no additional coding SNPs other than the ones obtained from the databases. Three coding nonsynonymous TLR8 SNPs, which were identified in the dbSNP and Ensembl databases (http://www.ncbi.nlm.nih.gov/projects/SNP/ and http://www.ensembl.org/), were determined in the study group by real-time PCR assays. Detailed methodologies for PCR, melting curve analysis, and mutagenesis are available online in appendix A.

Plasmids. Details on the plasmids encoding β-galactosidase, the ELAM NF-κB reporter plasmid (luciferase), and the human TLR8 isoforms are given online in appendix A.

Transient transfection of HEK 293 cells and measurement of luciferase activity. Transient transfection of HEK 293 cells and measurement of luciferase activity was performed as described in detail online in appendix A.

Isolation and stimulation of primary human blood cells. Whole blood was obtained at a fixed time in the morning from healthy volunteers, who had provided informed consent before donation. For each donor, the TLR8 A1G genotype was confirmed and the presence of the TLR8 Met10Val and Arg715Gln polymorphisms was excluded before blood collection. The detailed protocols for the isolation, culture, and stimulation of primary human blood cells are provided online in appendix A.

Cytokine and arachidonic acid metabolite measurement. Tumor necrosis factor (TNF)−α, interleukin (IL)−6, and IL-1 protein levels were measured in cell culture supernatants by a sandwich ELISA procedure, which is described in detail online in appendix A. After centrifugation (16,000 g for 10 s at 22°C), prostaglandin E2 (PGE2) levels in cell-free sample supernatants were analyzed by ELISA, in accordance with the manufacturer’s instructions (Cayman Chemical). Leukotriene B4 (LTB4) concentrations were analyzed by a liquid chromatography/mass spectrometry/mass spectrometry (LC-MS/MS) procedure, the details of which are provided online in appendix A.

Statistical methods. Genotype frequencies were compared between seropositive and seronegative individuals by the χ² test or by 2-tailed Fisher’s exact test if the χ² test was inappropriate. CD4+ T cell count declines over a minimum period of 6 months (including data from at least 2 study visits) were determined by linear regression analysis of each patient’s CD4+ T cell count between 1 month and 6 years after infection. Viral setpoints were defined as the median log_{10}-transformed copies of viral RNA per microliter of plasma between 100 days and 2 years after infection [16]. To assess the strength of the association between TLR8 A1G genotypes and disease progression, the individual CD4+ T cell count slopes as well as the viral setpoints were compared by the nonparametric Mann-Whitney U or Kruskall-Wallis test. For the analysis of the 497 seroconverters, the Kaplan-Meier method was applied. A CD4+ T cell count <200 cells/μL was
defined as the study end point. Differences between genotypes were tested by the log-rank test. \( P < .05 \) was considered to indicate statistical significance. SPSS (version 14.01) was used for data management and statistical analyses, and Prism (version 4) was used for figures.

**RESULTS**

**TLR8** is located on the X chromosome and spans \(~15.5\) kb (Xp22.3-p22.2). On the basis of sequence analysis, 2 transcript variants have been identified (figure 1A). The shorter transcript variant is transcribed from 2 exons of the gene and uses an initiator methionine located on exon 1, whereas the remainder of the protein is encoded on exon 2b. The corresponding protein (termed “isoform B”) is 1041 aa in length [17]. The longer variant includes an additional internal exon, resulting in the use of a start codon located on exon 2a. It encodes for a protein of 1059 aa (termed “isoform A” herein), with exon 2b being the major coding exon [18]. Isoform A has an extended N-terminus compared with isoform B, murine TLR8, and human/murine TLR7.

To assay the activity level of each receptor variant, we first compared the TLR8 isoforms by means of transfected HEK 293 cells (figure 1B). HEK 293 cells overexpressing isoform B were treated with the TLR7/8 ligand resiquimod, which induced dose-dependent NF-kB release as described elsewhere [4]. In contrast, TLR8 isoform A invoked a significantly lower NF-kB response on resiquimod stimulation, implying that TLR8 isoform B is the more active receptor variant. In a candidate gene approach, we thus chose to examine SNPs that affect isoform B.

A database search revealed the occurrence of 3 coding non-synonymous TLR8 polymorphisms. Two of them, TLR8 Met10Val (rs5744077) and TLR8 Arg715Gln (rs5744082) (numbering corresponds to isoform A amino acid positions), are located within the major coding exon, which is common to both isoform A and B. The third, referred to as TLR8 A1G (rs3764880; denotation is according to the nucleic acid change involved, since an initiator methionine is concerned) in the following, converts the isoform B start ATG into a GTG codon but does not affect isoform A coding exons (figure 1A).

We determined the frequencies of these TLR8 SNPs in a total of 782 HIV-infected patients and 550 control subjects to evaluate the influence the polymorphisms have on susceptibility to and progression of the HIV infection. We first performed genotype assessment for the 550 seronegative individuals, who were age and sex matched with the seropositive group. In the control group, TLR8 A1G was the most common genetic variation regardless whether the study subjects were white \((n = 515)\) or of African background \((n = 35)\). TLR8 Met10Val, which was detected in only 1 hemizygous white individual, was not rare among Africans, with an allele frequency of 17% in both men and women. As expected, the TLR8 Arg715Gln mutation, which has so far been reported exclusively in African Americans

![Figure 1](http://innateimmunity.net/IIPGA2/PGAs/InnateImmunity/TLR8/generateReport) at low frequencies, was completely absent in this mainly white study population. If the presence of a mutant allele exerts a biological advantage with respect to HIV susceptibility, its frequency should be decreased in HIV-infected populations. We therefore genotyped 782 seropositive individuals, and genotype frequencies were compared with those of the seronegative control subjects. The rare TLR8 Arg715Gln form
was not detected, and the frequency of the TLR8 Met10Val mutation could be compared only among African HIV-positive patients \( (n = 26) \) and control subjects \( (n = 35) \). No significant difference in genotype distribution was observed between the groups. In both white and African HIV-infected patients, frequencies of TLR8 A1G approximated those in seronegative individuals. We conclude that, for the examined SNPs, an affect on susceptibility to HIV infection is unlikely in white persons, of whom a sufficiently large sample number was studied (table 1).

We next wanted to know whether the frequent TLR8 G1 mutation was associated with HIV disease progression. When set-point viral loads between mutation carriers and wild-type allele carriers were compared using the nonparametric Mann-Whitney \( U \) test, no significant difference was found (see Methods for definition; data not shown). However, individuals carrying the G variant displayed a significantly slower loss of CD4\(^+\) T cells than did those carrying the wild-type A allele \( (P = .008) \). These differences remained significant when the analysis was restricted to white individuals \( (P = .005) \) and were most pronounced in male white persons \( (P = .002) \) (table 2). The protective effect of the TLR8 A1G mutation was also observed in the Kaplan-Meier analysis of 487 seroconverters, which demonstrated that SNP carriers reached the study end point of a CD4\(^+\) T cell count <200 cells/\( \mu \)L significantly later than did carriers of the normal gene variant (figure 2).

Because our data implied that the presence of the TLR8 G1 polymorphism is associated with a slower course of HIV infection, we elucidated its functional impact by means of in vitro and ex vivo cell stimulation experiments. We inserted the G1 mutation into the TLR8 isoform B expression plasmid, overexpressed the obtained TLR8 isoform B variant in HEK 293 cells, and stimulated these with resiquimod (figure 1C). The mutated TLR8 isoform B, which was still more active than the TLR8 isoform A, displayed a decreased release of NF-\( \kappa \)B compared with wild-type TLR8 isoform B, supporting the notion that this polymorphism has a functional effect on TLR8 activity.

The frequent occurrence of the TLR8 A1G variant in all ethnic groups provided the opportunity to study its role in humans ex vivo. To examine whether the presence of the mutation affects cytokine induction, we stimulated blood cells obtained from healthy consenting donors by means of resiquimod and HIV-derived ssRNA-40. Monocytes express more TLR8 and less TLR7 than do other peripheral blood mononuclear cell (PBMC) subsets [19]; therefore, we enriched the PBMCs for monocytes before stimulation. Individuals hemi- or homozygous for the G1 allele were compared with matched hemi- and homozygous carriers of the wild-type allele. On treatment with resiquimod, adherent cells from carriers of the mutated TLR8 variant displayed a significantly increased TNF-\( \alpha \) response \( (P = .012) \), whereas no such difference was observed for IL-6 secretion (figure 3A). A similar trend was noted when the monocyte-enriched PBMCs were treated with HIV-derived ssRNA-40. Consistent with published findings, the control ssRNA-41 did not induce the expression of any of the examined cytokines [4]. Contrary to TLR8 stimulation, lipopolysaccharide (LPS)–driven TLR4 stimulation led to similar levels of cytokine secretion for the 2 groups tested. Further experiments showed that the presence of the TLR8 G1 allele augmented the TNF-\( \alpha \) response in a time- as well as a dose-dependent fashion, whereas IL-6 levels remained comparable. Of note, the kinetics analysis also included 2 female individuals heterozygous for the TLR8 G1 allele, who displayed TNF-\( \alpha \) levels in between those of homozygous mutant and wild-type allele carriers (figure 3B).

For the anti-inflammatory cytokine IL-10, resiquimod at different concentrations invoked similar responses in both genotypes. However, resiquimod at 5 \( \mu \)mol/L led to a discrete difference in IL-10 release after 24 h of stimulation \( (P = .028) \). This

### Table 1. Genotype frequencies of nonsynonymous Toll-like receptor (TLR) 8 polymorphisms in HIV-positive individuals and control subjects.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No. (%) of subgroup</th>
<th>Mean (SE)</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/A or A/−</td>
<td>180 (76.3)</td>
<td>−7.26 (0.84)</td>
<td>.008</td>
</tr>
<tr>
<td>A/G</td>
<td>4 (1.7)</td>
<td>−4.70 (1.32)</td>
<td></td>
</tr>
<tr>
<td>G/G or G/−</td>
<td>52 (22.0)</td>
<td>−1.78 (1.55)</td>
<td></td>
</tr>
</tbody>
</table>

**NOTE.** Shown are the no. (out of 236 HIV-1–infected individuals) for whom CD4\(^+\) T cell count slopes were available, based on a cohort of 782 genotyped patients, the mean and SE of monthly CD4\(^+\) T cell count decline, and the respective \( P \) value calculated using either the Mann-Whitney \( U \) (male individuals) or the Kruskal-Wallis test.

\( a \) Because of the low case nos., analysis of disease progression in female patients \( (n = 14) \) was not conducted separately.

\( b \) Because of the low case nos. for Asians and South Americans with follow-up CD4\(^+\) T cell count data, these were not analyzed separately.

\( c \) “/−” denotes the absence of the particular allele in hemizygous males.

**Table 2. Association between Toll-like receptor (TLR) 8 A1G genotype and monthly loss of CD4\(^+\) T cells.**

<table>
<thead>
<tr>
<th>Sex, ethnicity,( a,b ) TLR8 A1G genotype( c )</th>
<th>No. (% of subgroup)</th>
<th>Mean (SE)</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combined</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A/A or A/−</td>
<td>180 (76.3)</td>
<td>−7.26 (0.84)</td>
<td>.008</td>
</tr>
<tr>
<td>A/G</td>
<td>4 (1.7)</td>
<td>−4.70 (1.32)</td>
<td></td>
</tr>
<tr>
<td>G/G or G/−</td>
<td>52 (22.0)</td>
<td>−1.78 (1.55)</td>
<td></td>
</tr>
<tr>
<td>White</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A/A or A/−</td>
<td>172 (77.5)</td>
<td>−7.50 (0.87)</td>
<td>.005</td>
</tr>
<tr>
<td>A/G</td>
<td>3 (1.4)</td>
<td>−5.49 (1.49)</td>
<td></td>
</tr>
<tr>
<td>G/G or G/−</td>
<td>47 (21.2)</td>
<td>−1.73 (1.61)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Combined</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A/−</td>
<td>170 (76.9)</td>
<td>−7.28 (0.88)</td>
<td>.004</td>
</tr>
<tr>
<td>G/−</td>
<td>51 (23.1)</td>
<td>−2.21 (1.52)</td>
<td></td>
</tr>
<tr>
<td>White</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A/−</td>
<td>164 (77.7)</td>
<td>−7.42 (0.91)</td>
<td>.002</td>
</tr>
<tr>
<td>G/−</td>
<td>47 (22.3)</td>
<td>−1.73 (1.61)</td>
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</table>
difference was contrary to that for TNF-α: on stimulation, cells from wild-type allele carriers displayed higher IL-10 levels than did those from SNP carriers (figure 3D). Resiquimod activates both TLR7 and TLR8. To verify that the observed difference in TNF-α and IL-10 secretion depended on TLR8, PBMCs were stimulated using resiquimod and the TLR7-specific ligand imiquimod. Imiquimod, at concentrations up to 500 μmol/L, induced the expression of IL-6 but not TNF-α and induced only negligible amounts of IL-10 (figure 4). For further verification, we conducted a pilot experiment involving a pair of healthy male volunteers differing in their TLR8 A1G genotype and another TLR stimulus: PBMCs were stimulated with CL075, a ligand with greater TLR8 specificity than resiquimod. We observed a similar trend, with higher TNF-α and lower IL-10 production for the hemizygous mutation carrier, confirming our previous results (figure 5).

We were able to show recently that the TLR8 signaling pathway is coupled to the lipid mediator network through stimulation of LTB4 and expression of the inducible cyclooxygenase (COX) isoform, COX-2 [20]. Therefore, we examined whether the presence of the TLR8 G1 SNP influences the priming effect of resiquimod toward polymorphonuclear neutrophils (PMNs). PMNs were isolated from donor pairs differing in their TLR8 A1G genotype and were pretreated with resiquimod (figure 6). PGE2, a representative of the COX-2–dependent class of lipid mediators, was measured following arachidonic acid stimulation. Neutrophils from hemizygous mutation carriers displayed a significantly higher enhancement of PGE2 biosynthesis than did those from wild-type carriers (P = .034) (figure 6A). Next, the effect of resiquimod on the 5-lipoxygenase product pathway was examined in wild-type and mutation carriers by assessing LTB4 production after formyl-Met-Leu-Phe (f-MLP) stimulation. LTB4 production was significantly higher in TLR8 G1 hemizygotes than in wild-type carriers, and this difference was even more pronounced than the difference observed for PGE2 production (P < .001).

**DISCUSSION**

It has been established during the last years that variations within the host’s genome contribute substantially to the individual course of an HIV-1 infection [21–24]. Our study represents the first report of a genetic TLR8 variant associated with restriction of HIV disease progression. It also confirms the importance of
TLRs in HIV disease observed in a recent study from Switzerland [13]. This study found that only 2 synonymous TLR9 SNPs occurred at higher frequencies in rapid than in normal progressors. Our study cohort, in contrast, contained a high proportion of seroconverter patients, which enabled us to detect a clear TLR8 A1G–related difference in progression to the study end point of a CD4⁺ T cell count < 200 cells/µL. Furthermore, comparison of the individual CD4⁺ T cell count declines by the Mann-Whitney U test uncovered an association that was apparently masked in the Swiss study, which was merely dichotomized according to rapid and normal CD4⁺ T cell losses.

Our functional results support the concept that TLR8 G1 is not a surrogate marker of other linked but unknown genes that

![Figure 3](https://example.com/figure3.png)

**Figure 3.** Differential responsiveness of monocytes from wild-type (wt) and A1G hemi/homozygous white individuals after Toll-like receptor (TLR) 8 stimulation. Adherent cells cultured at 3 × 10⁵ cells in 0.1 mL were treated with resiquimod, HIV-derived synthetic single-stranded RNA-40/41 complexed with DOTAP, or lipopolysaccharide under blinded conditions at 37°C. Cytokine levels in the supernatants were determined by ELISA. A, Tumor necrosis factor (TNF)-α and interleukin (IL)-6 response of wt and mutant donors after 16 h of stimulation. Horizontal bars represent mean titers. B, Kinetics for resiquimod stimulation at 1 µmol/L. C, Resiquimod dose-response curve for TLR8 stimulation. D, IL-10 response of wt and mutant donors after 24 h of stimulation at 5 µmol/L resiquimod, kinetics for 5 µmol/L resiquimod, and dose response at 24 h after stimulation. The experiment shown in panel B included 2 heterozygous female donors, for whom the observed trend was not significant (SE omitted for graphical clarity). Four independent experiments involving 4 pairs of healthy volunteers were performed in duplicate. Values are expressed as nanograms per milliliter and represent the mean ± SE. *P < .05 for the difference between mutant and wt control (Mann-Whitney U test).

![Figure 4](https://example.com/figure4.png)

**Figure 4.** Different tumor necrosis factor (TNF)-α (A), interleukin (IL)-6 (A), and interleukin (IL)-10 (B) responses after treatment with the Toll-like receptor (TLR) 7/8 agonist resiquimod and the selective TLR7 agonist imiquimod.

![Figure 5](https://example.com/figure5.png)

**Figure 5.** Differential response to stimulation with the selective Toll-like receptor (TLR) 8 agonist CL075 by healthy donors hemizygous for the wild-type and the mutant variant.
might influence progression of disease. However, the mechanism for the role TLR8 A1G plays in the delay of disease progression is not immediately obvious. It was recently suggested that the persistent state of immune activation found in HIV-infected patients is mediated directly by activation of TLR7/8 through HIV RNA [8]. The TLR8 A1G mutation results in a decrease in NF-κB release, as was demonstrated here in the overexpression assays. This could accordingly be responsible for a lower level of immune activation, explaining the slower natural course of disease in mutation carriers. Thus, our observations support the concept that HIV disease progression depends on TLR7/8-mediated immune activation.

In addition to the TLR8 overexpression assays, which have been suggested to represent an artificial experimental setting [25], we investigated the effect of the mutation in primary cells from healthy volunteers. We found a modulation of cytokine patterns in monocyte-enriched PBMCs, with higher TNF-α production and a trend toward lower IL-10 production. Both cytokines play a profound but complex role in the control of HIV. TNF-α, considered an activator of HIV-1 replication in latently infected cells, has repeatedly been demonstrated to inhibit viral replication in freshly infected monocytes, macrophages, and brain cell cultures [26–28]. TNF-α sensitizes chronically infected cells to lysis through NK cells and may thus have an additional protective effect on uninfected cells [29]. Studies of the influence of IL-10 on HIV-1 replication have led to divergent results, depending on experimental conditions [30, 31]. It has been proposed that increased serum levels of IL-10 mark the progression to AIDS [32]. IL-10 renders primary monocytes more susceptible to productive HIV infection [33]; in latently as well as acutely infected cells, IL-10, in combination with various other cytokines, was found to enhance HIV expression [26, 34]. Thus, the altered cytokine profile displayed by TLR8 G1 mutant cells may be directly responsible for the beneficial effect observed in mutation carriers. Future studies should examine the effect of mutant allele carriage on cytokine activation in different cell populations and should assess HIV replication patterns in cells of different genotypes.

Past studies examining the role played by PMNs and lipid mediators in HIV infection have focused on 2 aspects. First, functional deficiencies in PMNs of untreated HIV patients contribute directly to the development of opportunistic diseases during HIV infection [35]. Second, both prostaglandins and leukotrienes have been implicated in the suppression of HIV replication. PGE2 has been demonstrated in vitro to inhibit HIV replication in acutely and chronically infected cells via down-regulation of CCR5 and inhibition of HIV promoter activity [36]. LTB4 administration leads to an increase in α-defensins and macrophage inflammatory protein–1β expression in vivo, both of which have strong anti-HIV activity [37]. TLR8 activation is known to potently enhance PGE2 and LTB4 production on stimulation of PMNs [20]. We observed an impressive pronouncement of this priming effect in TLR8 G1 mutant PMNs, and it is possible that this adds to the restriction of HIV disease pathogenesis in mutant allele carriers.

The TLR8 A1G polymorphism alters the start ATG of TLR8 isoform B into a GTG triplet. A methionine located at position 4 of isoform B could be used as an alternate start codon, resulting in a truncated TLR8 (1038 vs. 1041 aa) that exhibits a shorter signal peptide. The functional effect of the mutation may be related to a more rapid decay of TLR8 mRNA or to a disadvantageous Kozak context of the position 4 methionine [38–42].

Figure 6. More pronounced resiquimod priming for lipid mediator production in Toll-like receptor (TLR) 8 A1G mutant carriers. Prewarmed polymorphonuclear neutrophils (PMNs; 5 × 10⁶ cells/mL) from 3 pairs of male donors differing in their TLR8 A1G genotypes were analyzed for prostaglandin E₂ (PGE₂) and leukotriene B₄ (LTB₄) biosynthesis. Following pretreatment with resiquimod for 1 h, PMNs were stimulated with 5 μmol/L arachidonic acid (AA) for 15 min, and PGE₂ production was measured by ELISA (A). PMNs were pretreated with resiquimod for 30 min and stimulated with 300 nmol/L formyl-Met-Leu-Phe (f-MLP) for 5 min; LTB₄ production was assessed by liquid chromatography/mass spectrometry/mass spectrometry (B). Data shown are mean ± SD values from 1 representative experiment of 3 independent experiments giving identical results, each performed in duplicate. *P < .05 and **P < .001 for the difference between all mutation carriers and all wild-type allele carriers, as determined from data pooled from all 3 experiments.
also, the difference in signal peptide length could affect protein function [41]. When overexpressed in the HEK 293 system, the TLR8 A1G mutation led to lower NF-κB induction after stimulation compared with wild-type TLR8, which was matched by a lesser induction of IL-10 in monocytes from individuals expressing this mutant. However, an increase in TNF-α, LTB4, and PGE2 production was observed, which cannot be directly explained by inhibition of the NF-κB axis. Further studies are needed to elucidate the molecular mechanisms of this potential contradiction. In general, secretion of both cytokines and chemokines are subject to complex inter- and intracellular interplay. For example, diminished secretion of IL-10 would reduce its potent inhibitory effect on proinflammatory cytokines and thus lead to greater TNF-α release. Regarding PGE2, the enzyme that limits its synthesis rate is COX-2, the expression of which depends not on NF-κB alone but on various other transcription factors as well, whereas LTB4 up-regulation is not linked to NF-κB at all. Thus, a decrease in NF-κB activation would not necessarily lead to a decrease in the induction of COX-2 or LTB4 expression [42, 43].

In conclusion, the data presented here demonstrate the protective effect of a TLR8 mutation on HIV disease progression in a German cohort of HIV-positive patients. We show that presence of the mutation leads to decreased NF-κB activation in vitro and has a strong impact on the production of different cytokine and lipid mediator classes, namely, TNF-α, IL-10, PGE2, and LTB4. The modulation of NF-κB release as well as the altered cytokine and lipid mediator production profiles are not only compatible with the restriction of HIV-1 infection but lead us to propose that future studies should address relevance of the TLR8 A1G polymorphism in the clinical course of other chronic and acute RNA viral diseases. The results of the present study may have implications for assessment of the individual patient’s risk profile as well as for the future use of TLR agonists in the prevention of or therapy for not only HIV-1 disease but potentially other viral diseases as well.

**PARTICIPATING COLLABORATORS**

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**References**


