

The role of TLR9 polymorphism in susceptibility to pulmonary tuberculosis

Deepak Bharti · Ashish Kumar · Ranjeet Singh Mahla · Sushil Kumar · Harshad Ingle · Hari Shankar · Beenu Joshi · Ashwin Ashok Raut · Himanshu Kumar

Received: 9 July 2014 / Accepted: 9 September 2014 / Published online: 25 September 2014
© Springer-Verlag Berlin Heidelberg 2014

Abstract *Mycobacterium tuberculosis* (MTB) is the causative agent of pulmonary tuberculosis (PTB), a major health problem that leads to 1.5 million deaths annually. Host genetic factors play a significant role in disease resistance/susceptibility by altering immunity against MTB. Toll-like receptor (TLR) sensors such as TLR2, TLR4, TLR8, and TLR9 are known to play a pivotal role in PTB via modulating sensor expression and/or effector responses. Single-nucleotide polymorphism (SNP) rs187084 (T-1486C) of the TLR9

promoter is associated with various autoimmune disorders and cancers. A recent bioinformatic analysis predicted that the T-1486C SNP is involved in PTB, although its potential role is unclear. To investigate the role of T-1486C in PTB, we stimulated PBMCs with the H37Rv whole cell lysate. We found that the presence of the “C” allele increases the transcriptional activity of the TLR9, which in turn induces high levels of Interferon gamma-induced protein 10 (IP-10), a biomarker for PTB. However, the expression of protective cytokines such as IFN γ and TNF α was observed significantly less with “C” allele in comparison to “T” allele. We further selected three different tribe populations showing differential susceptibility to PTB and performed genotypic analyses for the TLR9 promoter. We found a significantly lower minor allele frequency (MAF) of T-1486C in the Baiga tribe, wherein fewer PTB cases were reported, than that in the Gond and Korku tribes. Collectively, these data suggest that the minor “C” allele at rs187084 locus may be associated with susceptibility to PTB, which may explain the relatively lower PTB rates observed in Baiga tribe members.

Deepak Bharti, Ashish Kumar, and Ranjeet Singh Mahla contributed equally to this work.

Electronic supplementary material The online version of this article (doi:10.1007/s00251-014-0806-1) contains supplementary material, which is available to authorized users.

D. Bharti · A. Kumar · R. S. Mahla · S. Kumar · H. Ingle · H. Kumar
Laboratory of Immunology, Department of Biological Sciences,
Indian Institute of Science Education and Research (IISER),
Indore-Bypass Road, Bhuri, Bhopal 460023, Madhya Pradesh,
India

H. Shankar · B. Joshi
National JALMA Institute for Leprosy & Other Mycobacterial
Diseases, Agra, Uttar Pradesh, India

A. A. Raut
Pathogenomics Lab, OIE Reference lab for Avian Influenza,
National Institute of High Security Animal Diseases, Anand Nagar,
Bhopal 462022, Madhya Pradesh, India

H. Kumar
Laboratory of Host Defense, WPI Immunology Frontier Research
Center, Osaka University, Osaka, Japan

H. Kumar (✉)
Laboratory of Immunology, Department of Biological Sciences,
Indian Institute of Science Education and Research (IISER) Bhopal,
Indore-Bypass Road, Bhuri, Bhopal 462066, Madhya Pradesh,
India
e-mail: hkumar@iiserb.ac.in

Keywords Single-nucleotide polymorphism (SNP) · Pulmonary tuberculosis (PTB) · IP-10 · IFN γ · TNF α · Tribes

Introduction

Pulmonary tuberculosis (PTB) is a major worldwide health problem, with approximately one third of the global population (~2 billion people) reported to be infected with the acid-fast bacillus *Mycobacterium tuberculosis* (MTB). Although two thirds of the infected individuals are latently infected without any active disease, the remaining one third develop active tuberculosis infections, which are responsible for 1.5 million deaths globally per year (Davila et al. 2008; Ottenhoff and Kaufmann 2012; Zumla et al. 2013). Studies suggest that

socioeconomic, environmental, immunogenetic, and nutritional factors are responsible for the development of PTB (Bhargava et al. 2013; Boccia et al. 2011; Pothlichet and Quintana-Murci 2013; Schmidt 2008).

Innate immunity acts as the first line of defense against infectious agents via innate immune cells expressing pattern recognition receptors (PRRs). PRRs also play an important role in the development of microbe-specific adaptive immunity. Among the various families of PRRs, Toll-like receptors (TLRs) are essential sensors that detect a wide variety of molecular species in various cellular compartments (Harding and Boom 2010; Kleinnijenhuis et al. 2011). Human TLR3, TLR7, TLR8, and TLR9 (NM_017442) are primarily expressed in the endolysosomes of phagocytic immune cells where MTB resides and replicates during infection. Human TLR8 and TLR9 are known to activate innate immune responses against MTB and have been shown to play important roles in determining infection outcomes (Bafica et al. 2005; Davila et al. 2008) of TLR8, namely C-129G, A-2167G, and A-1145G, were found to be associated with PTB in Indonesian and Russian population, suggesting that SNPs in regulatory gene regions are critical in determining host susceptibility to PTB (Davila et al. 2008). Another endolysosomal localized TLRs, TLR3 and TLR7 sense RNA of RNA viruses, are not involved in limiting MTB infection (Kumar and Bot 2013).

The majority of immune cells such as plasmacytoid dendritic cells, monocytes, macrophages, neutrophils, natural killer (NK) cells, and B cells express TLR9 (Guillerey et al. 2012). Genetic studies with TLR9-deficient mice revealed the functional importance of TLR9 is non-redundant in PTB, after MTB infection via regulation of Th1 responses (Bafica et al. 2005). TLR9 recruits a sole adaptor, MyD88, which activates a cascade of downstream signaling events for production of inflammatory cytokines and type I interferons (IFNs) through transcription factors NF- κ B and IRF, respectively. Type I IFNs further induce type I IFN-inducible chemokines such as inducible protein 10 (IP-10) and RANTES.

The PTB occurrence at different rates among different populations indicates that host genetic factors play crucial roles in differential susceptibility. The SNPs in the regulatory element/promoter region of the TLR9 may play a direct role in PTB by affecting TLR9 expression levels. Several SNPs have been identified in the TLR9 promoter region, among all, rs187084 (T-1486C) and rs5743836 (T-1237C) SNPs, have been shown to be associated with various autoimmune disorders and cancers (Pothlichet and Quintana-Murci 2013; Tao et al. 2007); however, its role in PTB is currently unknown. Although, it has been predicted by using bio-informatics approach that rs187084 may be involved in tuberculosis.

In the present study, we performed functional analysis of the rs187084 C allele and found that rs187084 C allele elevates TLR9 expression, as well as production of IP-10 (a

biomarker for active PTB). However, the C allele of rs187084 was associated with decreased levels of IFN γ (a protective cytokine) in peripheral blood mononuclear cells following stimulation with H37Rv whole cell lysate (wcl). To further confirm the findings, we performed genotypic analysis of the TLR9 promoter region in the Baiga tribe, which can be relatively resistant to PTB compared to the Gond and Korku tribes (Yadav et al. 2010). We found that the rs187084 C allele is present at low frequency (18 %) among Baigas compared to global, Gond, and Korku tribes (40–42 %). Collectively, these results provide an evidence that rs187084 may be associated with PTB susceptibility.

Material and methods

Study populations

Three hundred individuals from three tribes, Baiga, Gond, and Korku (100 from each) of Central India belonging to Mandla and Betul districts of Madhya Pradesh (M.P.), were included in this study. Baiga, Gond, and Korku have a population size of 248,949; 534,988 and 66,781, respectively, in M.P. All experiments using blood samples were performed in accordance with relevant guidelines and regulation after approval from Institutional Ethical Committee (IEC), IISER Bhopal. Informed consent was also obtained from all the individuals/subjects.

Polymerase chain reaction and sequencing

Blood samples from the individuals were spotted on Whatman FTA Classic Cards (GE healthcare) and processed for polymerase chain reaction (PCR) as per manufacturer's instructions. The region containing the SNP (rs187084) was amplified using Phusion Blood Direct PCR Kit (Thermo scientific) as per manufacturer's protocol using primers (forward: GCCATGATACCACCCA and reverse: GCAGAGAGCAGGGCAGGACAG-3'). The sequencing of PCR product was performed using 3730 DNA Analyzer Applied Biosystems sequencer by using the same forward primer used in the PCR amplification. The SNP was then analyzed by using Sequencing Analysis v5.4.

Population frequencies

TLR9 SNP genotype and allele frequency were calculated manually using formulae given below:

Genotype Frequency

$$= \frac{\text{(Number of individuals having that particular genotype)}}{\text{(Total Number of individuals)}}$$

For example, $f(PP)=A/D$, $f(pp)=B/D$, $f(Pp)=C/D$, where A , B , C , and D represents number of individuals homozygous

for genotype PP , pp , heterozygous for genotype Pp , and total number of individuals, respectively.

Allele Frequency

$$= \frac{\{(2 \times \text{Number of individuals having genotype homozygous for that particular allele}) + (\text{Number of individuals having heterozygous genotype})\}}{(2 \times \text{Total Number of individuals})}$$

For example, $P=f(PP)+1/2f(Pp)$, $p=f(pp)+1/2f(Pp)$, where $f(PP)$, $f(pp)$, and $f(Pp)$ represent frequency of homozygous genotype PP , pp , and heterozygous Pp , respectively.

Hardy–Weinberg Equilibrium (HWE) consistency was determined by comparing observed number of different genotypes with those expected under the HWE by using formulae given below: $\text{Exp}(PP)=P^2n$, $\text{Exp}(Pp)=2pqn$, and $\text{Exp}(pp)=p^2n$, where P and p are two alleles. Pearson's chi-square: $\chi^2=\sum(O-E)^2/E$, where O and E represent observed and expected number of genotypes, respectively.

The probability value (p) was calculated by using 2×2 contingency chi-square test.

Cell culture

Peripheral blood mononuclear cells (PBMCs) from the blood of healthy tribe donors were isolated using Histopaque-1077 (Sigma) as per manufacturer's protocol. PBMCs were cultured in RPMI 1640 medium supplemented with 10 % fetal bovine serum after counting and testing viability using trypan blue exclusion method. Cells were plated at a density of $1 \times 10^6/200 \mu\text{l}$ in 96-well plates and stimulated with H37Rv wcl and incubated at 37 °C, 5 % CO_2 . Cells were harvested at 24 and 48 h following infection for RT-PCR and ELISA, respectively.

SYBR Green quantitative reverse transcription PCR

Total RNA was isolated using Trizol RNA isolation protocol. Two hundred nanograms of total RNA was used for complementary (c)DNA synthesis using iScript™ cDNA Synthesis Kit (Bio-Rad) which uses random hexamer primers. One microliter of the cDNA was used for the RT-PCR reaction. All RT-PCR reactions were performed using SYBR Green PCR Master Mix in StepOnePlus™ Real-time PCR System (Applied Biosystems) and the cycling conditions were composed of initial denaturation step at 95 °C for 10 min followed by 40 cycles at 95 °C for 15 s and 60 °C for 1 min. The experiments were carried out in triplicate for each gene. The housekeeping gene 28S was used as an internal control for normalization. The relative quantification in gene expression was determined using the $2^{-\Delta\Delta\text{CT}}$ method.

Flow cytometry

PBMCs were harvested and fixed using 1 % PFA for 15 min at RT. Fixation was blocked using 2 % fetal calf serum–phosphate-buffered saline (FCS-PBS), and then, cells were stored in 2 % FCS-PBS until further use. For intracellular staining of TLR9, cells were permeabilized using 0.1 % Triton-X 100 for 15 min at RT, washed twice with PBS, and incubated with antimouse TLR9 antibody (IMGENEX; IMG-305A) at dilution of $1 \mu\text{g}/10^6$ cells for 30 min. Cells were incubated with antimouse secondary antibody Alexa-Fluor-488 (A21202) for 30 min at RT, washed twice with PBS and reconstituted in 500 μl of PBS, and analyzed with BD FACS Aria III. PBMCs were gated against FITC channel and analyzed using FACS Diva software.

ELISA

ELISA for $\text{TNF}\alpha$, $\text{IFN}\gamma$, and IP-10 was performed according to manufacturer's instructions using human $\text{TNF}\alpha$ ELISA set (25833), human $\text{IFN}\gamma$ ELISA set (555142), and human IP-10 ELISA set (550926), respectively. Protocol procedures were carried out using BD OptEIA reagent set B (550534) in 96-well microtiter plate from BD (351172).

Statistical analysis

All the data was analyzed by GraphPad Prism 5 (USA). Two-tailed Student's unpaired t test was used to determine statistical significance ($p < 0.05$). All experiments were performed at least in triplicate.

Results

The “C” allele enhances transcription and translation of TLR9 after stimulation with *M. tuberculosis*

To investigate the functional consequences of “C” or “T” SNP variants in the TLR9 promoter at -1486 position during the development of PTB, the peripheral blood mononuclear cells

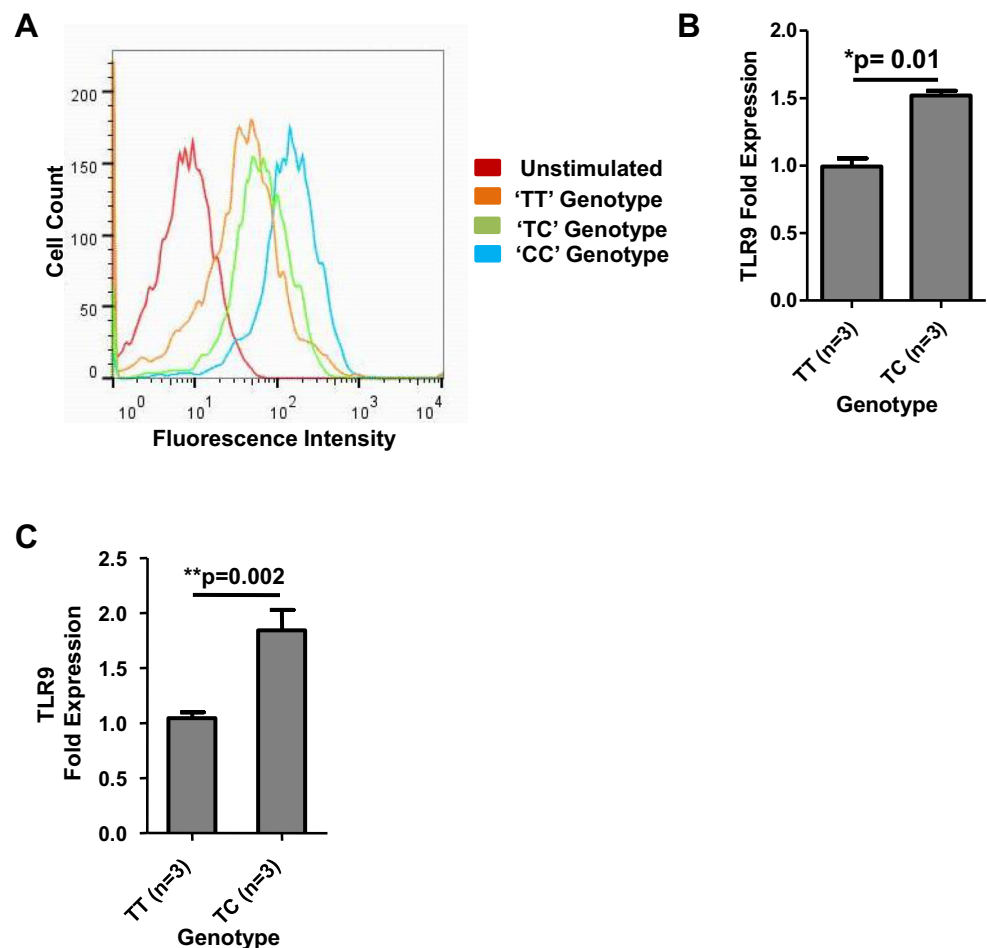
(PBMCs) from individuals with TT, TC, and CC genotypes were isolated and stimulated with heat-killed H37Rv wcl. To this end, we used flow cytometry to determine when TLR9 protein expression in PBMCs was maximal after stimulation with H37Rv wcl. We found that TLR9 expression in PBMCs reached a maximum at 48 h post-H37Rv wcl exposure (Figure S1). Subsequently, we tested TLR9 expression in PBMCs from individuals with different genotypes (TT=6; TC=5; and CC=3) and found that the CC and TT genotypes were associated with the highest and lowest expression levels, respectively (Fig. 1a). The TC genotype showed intermediate TLR9 expression at 48 h (Fig. 1a). Next, we examined TLR9 mRNA expression associated with TT and TC genotypes by using quantitative reverse-transcriptase PCR (qPCR). TLR9 expression was markedly higher ($p=0.01$), in individuals with TC vs. TT genotypes (Fig. 1b). To confirm the differential TLR9 expression observed with different genotypes, PBMCs from individuals with TT and TC genotypes were stimulated with H37Rv wcl for 48 h and visualized by confocal microscopy. Images were analyzed using ImageJ software and showed that the fluorescence intensity in TT genotypes was significantly reduced ($p=0.002$) compared to TC genotypes

(Fig. 1c). It is noteworthy that the numbers of individuals with the CC genotype are very low in the populations studied; therefore, we could not include CC genotype samples in the qPCR or confocal microscopy experiments. Collectively, these observations demonstrate that the “C” allele results in increased transcriptional activity of TLR9 and TLR9 expressions is related to genotypes in the order $TT < TC < CC$, which may relate to PTB susceptibility/resistance.

The “C” allele induces significantly higher expression of the PTB biomarker, IP-10

To investigate whether increased TLR9 expression is associated with PTB, we examined expression of the type I IFN-inducible protein IP-10 (CXCL10), which is a well-known biomarker for PTB (Aabye et al. 2013; Hong et al. 2014; Ruhwald et al. 2012). We stimulated PBMCs with H37Rv wcl from individuals with TT ($n=5$) and TC ($n=5$) genotypes, and the expression of IP-10 transcripts was quantified by qPCR. We observed that IP-10 transcript levels were significantly higher with the TC genotype, relative to TT individuals ($p=0.0043$; Fig. 2a). We further

Fig 1 Minor Allele enhances the transcriptional activity of TLR9. **a** Human PBMCs with TT, TC, and CC genotypes were stimulated with MTB H37Rv wcl for 48 h and stained for TLR9. The TLR9-positive cells were measured by flow cytometer. The results are representative of five individuals with TT and TC genotypes. The TLR9 expression for CC is representative of two CC genotype individuals. **b** Human PBMCs (peripheral blood mononucleated cells) with genotypes TT and TC were stimulated with or without H37Rv wcl. The expression of TLR9 was measured by quantitative PCR (qPCR) and normalized to the expression of 28S. Un-stimulated cells were considered to be 1. The results are representative of three individuals for each genotype. $*p=0.01$, calculated by *t* test. **c** Cells used in flow cytometer were processed for confocal microscopy, and relative fold (RF) for TLR9 expression was calculated. Un-stimulated cells were considered to be 1. $**p=0.002$, calculated by *t* test



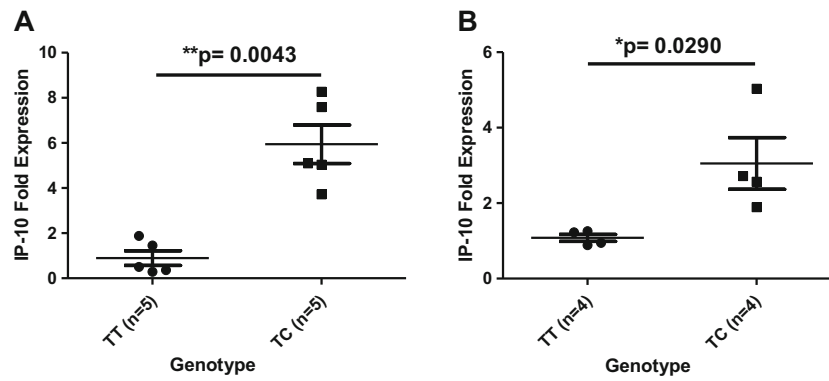


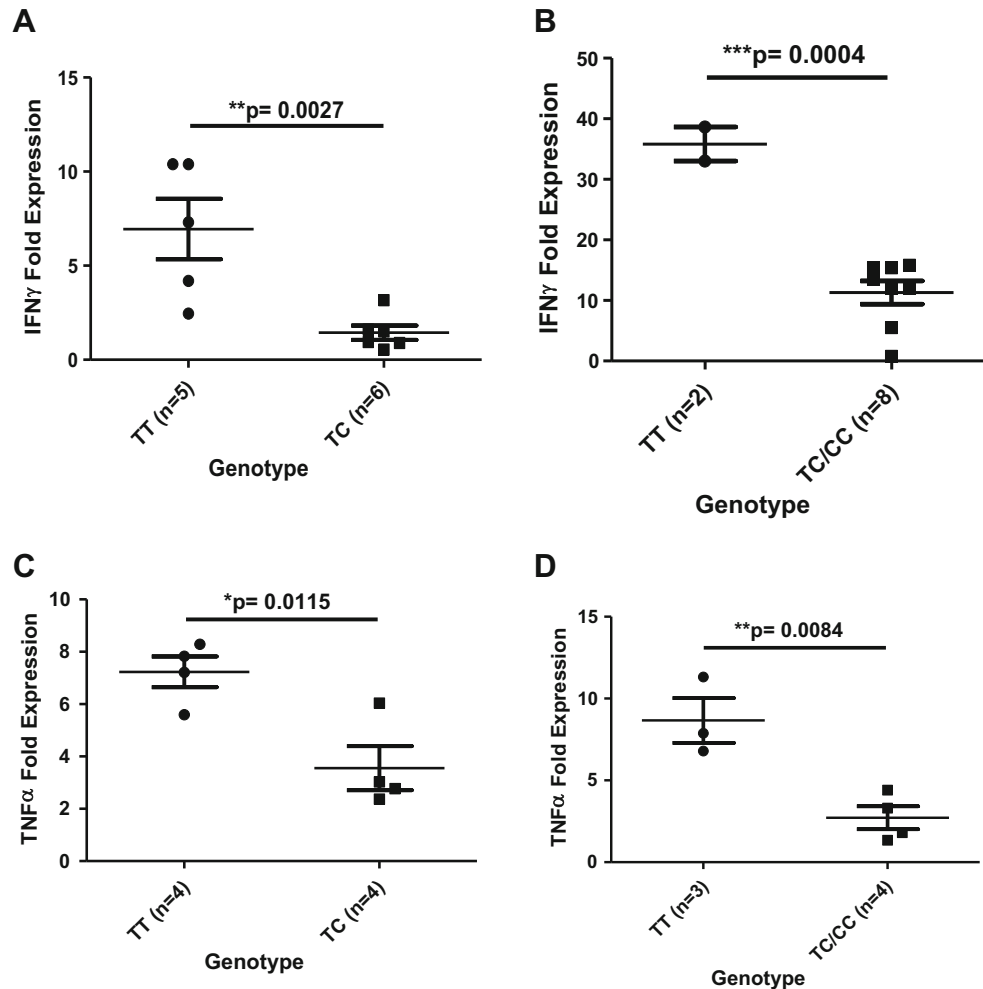
Fig 2 Increase of IP-10, a marker for active tuberculosis cytokine in “TC” compared to “TT” individuals. PBMCs from **a** TT (*n*=5) and TC (*n*=5) and **(b)** TT (*n*=4) and TC (*n*=4) genotype individuals were

stimulated with H37Rv wcl or left un-stimulated. After 12 h, IP-10 mRNA and protein expressions were quantified by **a** qPCR and **b** ELISA, respectively. *******p*=0.0043 (**a**) and ******p*=0.0290 (**b**), calculated by *t* test

confirmed the observation by measuring the production of IP-10 by ELISA following stimulation of PBMCs from different genotypes with H37Rv wcl. We observed that IP-10 production was significantly higher in TC, relative to TT individuals (*p*=0.0290), consistent with

observations relating to IP-10 mRNA expression (Fig. 2b). Collectively, these results indicate that the number of C alleles in the TLR9 promoter at position -1486 is directly linked to TLR9 expression and IP-10 production and may influence the development of PTB.

Fig 3 Increase of IFN γ and TNF α , a protective cytokine against PTB in “TT,” compared to “TC” individuals. PBMCs from **a** TT (*n*=5) and TC (*n*=6), **b** TT (*n*=2) and TC (*n*=6)/CC (*n*=2), **c** TT (*n*=4) and TC (*n*=4), and **d** TT (*n*=3), TC (*n*=3)/CC (*n*=1) genotype individuals were stimulated with MTB H37Rv wcl or left un-stimulated. After 12 h, **a** IFN γ and **c** TNF α mRNA for and **b** IFN γ and **d** TNF α in culture supernatant were quantified by qPCR and ELISA, respectively. The *p*=0.0027 (**a**), *p*=0.0017 (**b**), *p*=0.0115 (**c**), and *p*=0.0084 (**d**) were calculated by *t* test



The “C” allele of rs187084 induces significantly low expression of IFN γ and TNF α , protective cytokines against PTB

The IFN γ , and the proinflammatory cytokine TNF α play a pivotal role in MTB clearance. These cytokines activate alveolar macrophages and promote efficient clearance of MTB in phagolysosome to overcome infection. Therefore, we stimulated PBMCs from individuals with the different genotypes with H37Rv wcl and examined expression of IFN γ and tumor necrosis factor (TNF α) mRNAs by qPCR. We observed significantly higher IFN γ and TNF α mRNA expressions in individuals with the TT genotype than in those with TC genotype ($p=0.0027$, $p=0.0115$; Fig. 3a, c). Production of IFN γ and TNF α in culture supernatants were also significantly higher ($p=0.0017$, $p=0.0084$) in TT genotype than in TC or CC genotype individuals as tested by ELISA (Fig. 3b, d). It is noteworthy that the TC and CC genotypes showed the same levels of IFN γ and TNF α in ELISA. This might be due to the decisive role of even a single T to C allele substitution. Thus, our results demonstrate that the “C” allele which enhances TLR9 expression and TLR9-dependent responses and particularly type I IFN-dependent responses that is IP-10 induces less production of IFN γ and TNF α and promotes PTB development.

Frequency of the minor “C” allele of rs187084 is significantly less in the comparatively PTB-resistant tribe

To test the hypothesis that SNPs in the promoter region of TLR9 may affect PTB susceptibility, we PCR-amplified and sequenced a region harboring rs187084 SNP by using samples from 100 individuals from the Baiga, Gond, and Korku tribes of Central India. The population sizes of the indicated tribes are approximately 534,988, 248,949, and 66,781, respectively. Sequencing was performed using primers that flanked base coordinates 52,260,689–52,261,463. Genotype and allele frequencies were calculated using the sequencing data. The minor allele frequencies (MAFs) of rs187084 were found to be 0.18, 0.41, and 0.42 for the Baiga, Gond, and Korku tribes, respectively (Fig. 4). The tribes were found in Hardy–Weinberg Equilibrium (Table S1). We found that the frequency of “C” allele of rs187084 for the Baiga tribe members (18 %) was significantly lower than those for the global, Gond, and Korku tribes (40–42 %; $p<0.001$ in each case).

Collectively, this observation suggests that rs187084 may be linked to the low prevalence of PTB in Baiga tribe (146/100,000) relative to the non-tribal Indian populations (186/100,000). If such a causal association exists, it is indicative of the fact that individuals with the “C” allele are more susceptible to PTB than individuals with “T” allele of rs187084, which may be explained by differential induction of transcriptional activity of TLR9 via the creation or disruption of

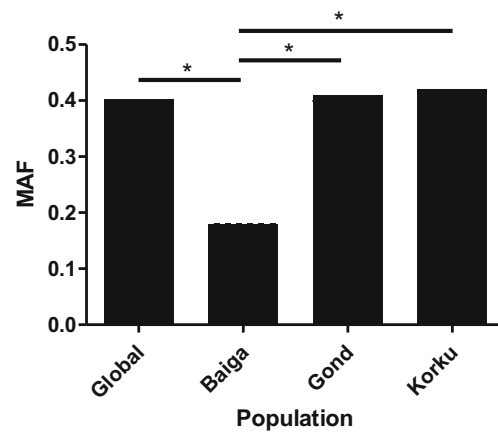


Fig. 4 Minor allele frequency at -1486 (rs187084) of TLR9 promoter is significantly less in Baiga compared to other tribes and global frequency. Comparison of MAF (minor allele frequency) between Baiga, Gond, Korku tribes and global (<http://www.ncbi.nlm.nih.gov/snp/>). Asterisks represent $p<0.001$, calculated by chi-square test

transcription factor-binding sites in its promoter region (Carvalho et al. 2011).

Discussion

TLR9 plays a pivotal role in PTB via modulating sensor expression and/or effector responses. Genetic variation in TLR9 gene can modulate the PTB susceptibility/resistance. Sequencing of the promoter region of TLR9 revealed the higher frequency of TT genotype in Baiga tribe demonstrating that relative frequency of the “C” allele is low in comparatively resistant Baiga tribe compared to frequencies observed in the phylogenetically similar Gond and Korku. We showed that the expression of TLR9 is inducible in PBMCs after stimulation with H37Rv wcl and those individuals having the “C” allele show increased transcriptional activity of TLR9. Increase in TLR9 expression results in enhancement of the type I IFN-inducible gene IP-10, which is a biomarker for active PTB. Recently, it has been shown that type I IFNs, which play an indispensable role against viral disease, down-regulate the production of protective cytokines such as TNF α and type II IFN (IFN γ) and IL-1 β during mycobacterial infection (Novikov et al. 2011; Teles et al. 2013). TNF α is produced by several types of innate immune cells such as monocytes, macrophages, and dendritic cells (Lin et al. 2007), whereas IFN γ is produced by both innate and adaptive immune cells such as monocytes, NK cells, neutrophils, T lymphocytes, and B lymphocytes (Bao et al. 2014; Matsumura et al. 2012). These cytokines play pivotal roles in macrophage activation during the elimination of intracellular MTB (Harding and Boom 2010). Baiga tribe possesses a low frequency of the “C” allele, which results in reduced

expression of TLR9 and IP-10 individuals; however, these individuals induced high IFN γ and TNF α expressions, which inhibit the intracellular replication of MTB and development of PTB. In contrast, Gond and Korku tribes and global populations show relatively high prevalence of PTB as well as high frequencies of the “C” allele. In addition to TLR9, there are likely to be several other genetic factors that may influence the development of PTB; many of which have already been described in previous studies and literature. Also, several other unknown factors, which need to be further investigated to understand MTB susceptibility, develop better diagnostic tools and improved molecular strategies for controlling MTB infection. Thus, one concludes that TLR9 T-1486C polymorphism is one of the several factors which may decide the degree of susceptibility of an individual towards tuberculosis.

Additionally, our study also suggests that therapeutic intervention of PTB can be achieved through inhibition of the expression of type I IFN and the type I IFN-inducible gene IP-10, which enhances the production IFN γ . These pharmacological blockers are not only important for PTB intervention but may also be helpful in the treatment of drug-resistant MTB or other intracellular bacterial infections.

Acknowledgments Authors would like to thank the sequencing facility of Department of Biological Sciences, IISER Bhopal. D.B. would like to thank for PDF support from IISER Bhopal. We would also like to thank Raunaq Singh Nagi for reading our manuscript and for helpful suggestions. This work is supported by research grants number SR/S2/RJN-55/2009 and BT/PR6009/GBD/27/382/2012 from Department of Science and Technology (DST) and Department of Biotechnology (DBT), Government of India (H.K.); MPCST3657/CST/BTA (D.B.) and Intramural Research Grant of IISER, Bhopal, India.

Ethical standards Authors declare that the experiments comply with the current laws of the country in which they were performed.

Conflict of interest Authors do not have any conflict of interest.

References

- Aabye MG, Latorre I, Diaz J, Maldonado J, Mialdea I, Eugen-Olsen J, Ravn P, Dominguez J, Ruhwald M (2013) Dried plasma spots in the diagnosis of tuberculosis: IP-10 release assay on filter paper. *Eur Respir J* 42:495–503
- Bafica A, Scanga CA, Feng CG, Leifer C, Cheever A, Sher A (2005) TLR9 regulates Th1 responses and cooperates with TLR2 in mediating optimal resistance to *Mycobacterium tuberculosis*. *J Exp Med* 202:1715–1724
- Bao Y, Liu X, Han C, Xu S, Xie B, Zhang Q, Gu Y, Hou J, Qian L, Qian C, Han H, Cao X (2014) Identification of IFN-gamma-producing innate B cells. *Cell Res* 24:161–176
- Bhargava A, Chatterjee M, Jain Y, Chatterjee B, Kataria A, Bhargava M, Kataria R, D'Souza R, Jain R, Benedetti A, Pai M, Menzies D (2013) Nutritional status of adult patients with pulmonary tuberculosis in rural central India and its association with mortality. *PLoS One* 8:e77979
- Boccia D, Hargreaves J, De Stavola BL, Fielding K, Schaap A, Godfrey-Faussett P, Ayles H (2011) The association between household socioeconomic position and prevalent tuberculosis in Zambia: a case-control study. *PLoS One* 6:e20824
- Carvalho A, Osorio NS, Saraiva M, Cunha C, Almeida AJ, Teixeira-Coelho M, Ludovico P, Pedrosa J, Pitzurra L, Aversa F, Romani L, Castro AG, Rodrigues F (2011) The C allele of rs5743836 polymorphism in the human TLR9 promoter links IL-6 and TLR9 up-regulation and confers increased B-cell proliferation. *PLoS One* 6:e28256
- Davila S, Hibberd ML, Hari Dass R, Wong HE, Sahiratmadja E, Bonnard C, Alisjahbana B, Szeszko JS, Balabanova Y, Drobniowski F, van Crevel R, van de Vosse E, Nejentsev S, Ottenhoff TH, Seielstad M (2008) Genetic association and expression studies indicate a role of toll-like receptor 8 in pulmonary tuberculosis. *PLoS Genet* 4:e1000218
- Guillerey C, Mouries J, Polo G, Doyen N, Law HK, Chan S, Kastner P, Leclerc C, Dadaglio G (2012) Pivotal role of plasmacytoid dendritic cells in inflammation and NK-cell responses after TLR9 triggering in mice. *Blood* 120:90–99
- Harding CV, Boom WH (2010) Regulation of antigen presentation by *Mycobacterium tuberculosis*: a role for Toll-like receptors. *Nat Rev Microbiol* 8:296–307
- Hong JY, Lee HJ, Kim SY, Chung KS, Kim EY, Jung JY, Park MS, Kim YS, Kim SK, Chang J, Cho SN, Kang YA (2014) Efficacy of IP-10 as a biomarker for monitoring tuberculosis treatment. *J Infect* 68:252–258
- Kleinnijenhuis J, Oosting M, Joosten LA, Netea MG, Van Crevel R (2011) Innate immune recognition of *Mycobacterium tuberculosis*. *Clin Dev Immunol* 2011:405310
- Kumar H, Bot A (2013) Innate immune recognition mechanisms and translational opportunities. *Int Rev Immunol* 32:113–115
- Lin PL, Plessner HL, Voitenok NN, Flynn JL (2007) Tumor necrosis factor and tuberculosis. *J Investig Dermatol Symp Proc* 12:22–25
- Matsumura T, Ato M, Ikebe T, Ohnishi M, Watanabe H, Kobayashi K (2012) Interferon-gamma-producing immature myeloid cells confer protection against severe invasive group A *Streptococcus* infections. *Nat Commun* 3:678
- Novikov A, Cardone M, Thompson R, Shenderov K, Kirschman KD, Mayer-Barber KD, Myers TG, Rabin RL, Trinchieri G, Sher A, Feng CG (2011) *Mycobacterium tuberculosis* triggers host type I IFN signaling to regulate IL-1 β production in human macrophages. *J Immunol* 187:2540–2547
- Ottenhoff TH, Kaufmann SH (2012) Vaccines against tuberculosis: where are we and where do we need to go? *PLoS Pathog* 8:e1002607
- Pothlichet J, Quintana-Murci L (2013) The genetics of innate immunity sensors and human disease. *Int Rev Immunol* 32:157–208
- Ruhwald M, Aabye MG, Ravn P (2012) IP-10 release assays in the diagnosis of tuberculosis infection: current status and future directions. *Expert Rev Mol Diagn* 12:175–187
- Schmidt CW (2008) Linking TB and the environment: an overlooked mitigation strategy. *Environ Health Perspect* 116:A478–A485
- Tao K, Fujii M, Tsukumo S, Maekawa Y, Kishihara K, Kimoto Y, Horiuchi T, Hisaeda H, Akira S, Kagami S, Yasutomo K (2007) Genetic variations of Toll-like receptor 9 predispose to systemic lupus erythematosus in Japanese population. *Ann Rheum Dis* 66:905–909
- Teles RM, Graeber TG, Krutzik SR, Montoya D, Schenk M, Lee DJ, Komisopoulou E, Kelly-Scumpia K, Chun R, Iyer SS, Sarno EN, Rea TH, Hewison M, Adams JS, Popper SJ, Relman DA, Stenger S, Bloom BR, Cheng G, Modlin RL (2013) Type I interferon suppresses type II interferon-triggered human anti-mycobacterial responses. *Science* 339:1448–1453
- Yadav R, Rao VG, Bhat J, Gopi PG, Selvakumar N, Wares DF (2010) Prevalence of pulmonary tuberculosis amongst the Baigas—a primitive tribe of Madhya Pradesh, Central India. *Indian J Tuberc* 57:114–116
- Zumla A, George A, Sharma V, Herbert N, Baroness Masham of Ilton (2013) WHO's 2013 global report on tuberculosis: successes, threats, and opportunities. *Lancet* 382:1765–1767