

Promoter polymorphism of *IL-8* gene and *IL-8* production in pulmonary tuberculosis

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Interleukin-8 (IL-8) is a chemokine which functions as a potent chemo attractant for the recruitment of leucocytes to the inflammatory sites. A polymorphism in position -251 in the promoter region of the *IL-8* gene has been shown to be associated with altered *IL-8* production. *IL-8*-251A promoter polymorphism was studied in 127 pulmonary tuberculosis (PTB) patients and 124 normal healthy subjects (NHS). *IL-8* gene variants were correlated with *IL-8* levels from peripheral blood mononuclear cells stimulated with phytohaemagglutinin, culture filtrate antigen (CFA) of *Mycobacterium tuberculosis* and live *M. tuberculosis*. No difference was observed in the variant genotype frequencies of *IL-8* gene and *IL-8* levels between NHS and PTB patients. NHS positive for TT genotype showed a higher spontaneous *IL-8* production than AA genotype ($P = 0.05$). Similarly, PTB patients with TT genotype showed significantly higher *IL-8* production to CFA ($P = 0.009$) and live *M. tuberculosis* ($P = 0.022$), compared to patients with AA genotype. The study suggests that the variant genotypes of -251 promoter polymorphism of the *IL-8* gene are not associated with susceptibility to PTB. Probably, TT genotype may be associated with higher *IL-8* production and increased leucocyte accumulation and inflammation at the site of *M. tuberculosis* infection.

Keywords: *IL-8*, *IL-8* level, promoter polymorphism, pulmonary tuberculosis.

MYCOBACTERIUM tuberculosis is a successful intracellular pathogen that can persist in the human host in the face of a robust immune response. Cytokines and chemokines produced by the various cellular components play a major role in immunity to tuberculosis (TB). Interleukin-8 (*IL-8*) is a chemokine belonging to the family of CXC cytokines produced by a variety of cell types in response to other inflammatory stimuli and recruits leucocytes to the areas of inflammation^{1,2}. Elevated *IL-8* levels have been shown in tuberculous pleural exudate, bronchoalveolar lavage and cerebrospinal fluids³⁻⁵. *IL-8* is expressed predominantly in tuberculous granulomas heavily infiltrated by neutrophils⁶. Recent studies have shown up-regulated *IL-8* gene expression in human macrophages infected with *M. tuberculosis*, which comprises part of the macrophage activation programme^{7,8}. A single nucleotide polymor-

phism in the *IL-8* promoter at position-251 relative to the transcription start site has been shown to be associated with susceptibility to TB⁹. However, a case-control study carried out in the Gambian TB patients revealed no association of *IL-8* -251 polymorphism with susceptibility to TB¹⁰. The *IL-8* -251A allele tends to be associated with increased production of *IL-8* by LPS stimulated whole blood as shown in an *in vitro* study¹¹.

The aim of the present study was to determine the regulatory role played by variant genotypes of promoter polymorphism of *IL-8* gene on *IL-8* production in response to live *M. tuberculosis* and culture filtrate antigen of *M. tuberculosis* under *in vitro* condition.

The study group consisted of 127 pulmonary tuberculosis (PTB) patients (mean age \pm SD: 36 ± 12.3) and 124 normal healthy subjects (NHS) (mean age \pm SD: 30 ± 9.1). Patients attending Tuberculosis Research Centre (TRC), Chennai with respiratory symptoms and radiographic abnormalities suggestive of PTB and sputum positive for *M. tuberculosis* by both smear and culture were included. Among the 127 patients, 73 were males and 54 females. Blood samples were collected before chemotherapy was started. NHS were volunteers who were clinically normal at the time of blood collection. Among the 124 NHS, 72 were males and 52 females. Patients and normals were of the same ethnic origin.

Twenty millilitre venous blood was collected, defibrinated and the peripheral blood mononuclear cells (PBMCs) were separated by Ficoll-Hypaque density gradient centrifugation¹². The PBMCs obtained were checked for viability and used for cytokine culture. DNA was extracted from a portion of lymphocytes as described earlier¹³ and concentration and purity of DNA were estimated spectrophotometrically. Promoter polymorphism (251 T A) of the *IL-8* gene was carried out as described earlier¹¹.

PBMCs were cultured at a concentration of 2×10^6 cells/ml in RPMI medium with 2% autologous serum for 72 h at 37°C and 5% CO₂. Cells were stimulated either with phytohaemagglutinin (PHA) (1 µg/ml), culture filtrate antigen (CFA) of *M. tuberculosis* (10 µg/ml) or live *M. tuberculosis* at a multiplicity of infection, 1:10. The culture supernatants were harvested at the end of 72 h and *IL-8* levels were estimated in the supernatants using commercially available ELISA kits (R&D Systems, USA).

Allele and genotype frequencies were determined by direct count. Pearson χ^2 -test was used to find out whether the genotype frequencies are in Hardy-Weinberg equilibrium. The 2×2 table, odds ratio and 95% confidence intervals were analysed using Epi Info Version 6.04 (CDC, Atlanta, GA, July 1996). Results of *IL-8* estimation are expressed as arithmetic mean \pm standard error (SE). Significance was tested using Student's *t* test. *P* value less than 0.05 was considered statistically significant.

The allele and genotype frequencies of -251 promoter polymorphism of *IL-8* gene are presented in Table 1. The frequency of allele 'A' was lower than allele 'T' in NHS

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as well as PTB patients. Genotype frequencies did not differ between NHS and PTB patients. However, genotype frequencies of NHS and PTB patients were significantly deviated from Hardy–Weinberg equilibrium (NHS: $P = 0.01$; PTB: $P = 0.0005$).

There was no significant difference in the total spontaneous and total stimulated IL-8 levels between NHS and PTB patients (data not shown). NHS with IL-8 TT genotype showed significantly higher spontaneous production

Table 1. Variant allele and genotype frequencies of *IL-8* gene –251 promoter polymorphism in normal healthy subjects (NHS) and pulmonary tuberculosis (PTB) patients

IL-8-251 Alleles and genotypes	NHS ($n = 124$)	PTB ($n = 127$)
Allele frequency		
Allele		
A	0.48	0.46
T	0.52	0.54
Genotype frequency (%)		
Genotype		
AA	16.9 (21)	12.6 (16)
AT	61.3 (76)	66.1 (84)
TT	21.8 (27)	21.3 (27)

n , Number of subjects; Numbers in parentheses represent subjects positive for each genotype.

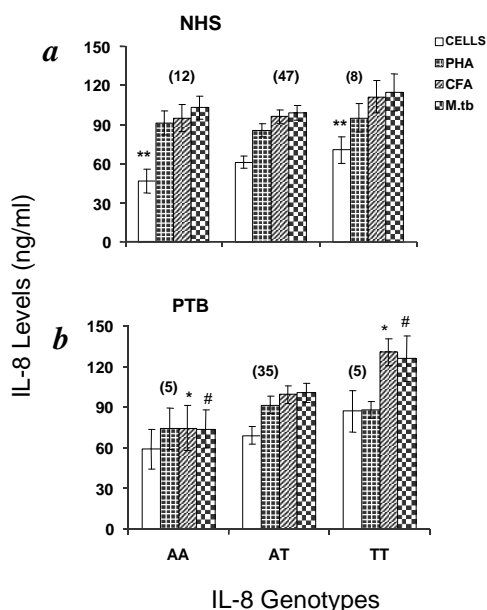


Figure 1. Spontaneous and induced production of IL-8 by PBMCs of NHS (a) and PTB (b) patients positive for variant IL-8 genotypes under *in vitro* culture conditions. PBMCs were cultured either without any agonist to determine spontaneous IL-8 release or stimulated with PHA, CFA or live *M. tuberculosis* for 72 h. Results are expressed as arithmetic mean \pm SE. Numbers in parentheses represent subjects studied. In NHS, TT genotype is associated with higher level of spontaneous IL-8 production than AA genotype (** $P = 0.046$). In PTB, TT genotype is associated with increased production of IL-8 in cultures stimulated with CFA (* $P = 0.01$) and *M. tuberculosis* ($\#P = 0.02$) compared to AA genotype.

of IL-8 compared to AA genotype ($P = 0.046$; Figure 1 a). Increased IL-8 production by PBMCs stimulated with CFA and live *M. tuberculosis* was observed in PTB patients with TT genotype than AA genotype. (CFA; $P = 0.01$; *M. tuberculosis*, $P = 0.02$; Figure 1 b).

We studied the IL-8-251 T A promoter polymorphism and *in vitro* IL-8 production in PTB patients and NHS. In the present study, the allele and genotype frequencies are similar to those of white subjects^{9,10}. On the contrary, an increased frequency of AA genotype and decreased frequency of TT genotype have been observed in African Americans and Gambians^{10,11}. This may be due to the environment–gene interaction.

A recent study by Ma *et al.*⁹ has suggested an association of IL-8-251A allele with increased TB risk for its carrier with a dominant mode. They have also suggested a linkage between IL-8 locus and TB susceptibility by showing preferential transmission of IL-8-251A allele to the affected children⁹. Even though the frequencies of the variant genotypes are similar to those of Whites, AA genotype is not associated with susceptibility to TB in Indian subjects. A significant association of the TT genotype with increased IL-8 production and comparatively decreased IL-8 production with AA genotype was observed with CFA and *M. tuberculosis*-stimulated cultures in patients. In NHS, TT genotype is significantly associated with increased spontaneous IL-8 production when compared to AA genotype. This is in contrary to an earlier study, which showed a significant association of the IL-8-251A allele with higher IL-8 levels *in vitro*¹¹. However, analysis in UK families revealed two major haplotypes containing the –251A allele, only one of which is associated with disease and increased IL-8 production. It suggests that the –251A allele may not be the functional one and it may be in linkage disequilibrium with a functional variant elsewhere in the *IL-8* gene¹⁴. Moreover, the reporter plasmid constructs containing 1409 base pairs of the 5' flanking region of *IL-8* gene differing only at the –251 position transfected into A549 cells, showed a higher expression for (–251T) than (–251A) constructs when stimulated with TNF *in vitro*¹⁵. These findings suggest that the –251T allele, in association with other functional variants in the *IL-8* gene, may be involved in higher IL-8 production in the Indian population.

IL-8 is a proinflammatory cytokine, which attracts neutrophils and lymphocytes to the site of infection. Polymorphisms in the *IL-8* gene are associated with increased IL-8 expression, which may lead to severe tissue damage in TB by increased cell recruitment and inflammation.

The present study suggests that variant genotypes of the promoter region of the *IL-8* gene are not associated with susceptibility to PTB in India. Probably, TT genotype in combination with other functional polymorphisms leads to higher IL-8 level, which may be associated with an increased leucocyte infiltration at the site of *M. tuberculosis* infection leading to an increased inflammatory res-

ponse. Further analysis of the *IL-8* gene may help identify more functional polymorphisms associated with altered *IL-8* production and susceptibility to TB in the Indian population.

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Breakdown of plantation residues by pill millipedes (*Arthrosphaera magna*) and assessment of compost quality

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We employed pill millipedes (*Arthrosphaera magna*) to generate compost from plantation crop residues on a pilot-scale. Three combinations of residues (w/w), viz. areca leaf litter and areca nut husk (1 : 1), cocoa leaf litter and cocoa pod husk (1 : 1) and mixed leaf litter (areca, acacia, cocoa and cashew) (1 : 1 : 1 : 1) in cement tanks were offered to millipedes with adequate moisture up to two months for composting. Particles less than 5 mm of millipede compost weighed about five times higher than control treatments. Total nitrogen ($P = 0.004$), phosphate ($P = 0.0006$) and C/N ratio ($P = 1.19 \times 10^{-6}$) significantly differed between control and treated residues. Organic matter and C/N ratio substantially declined in treated than in control residues. In areca compost, moisture and total nitrogen were elevated and pH was shifted from acidic to neutral. In mixed litter compost, phosphate, calcium and magnesium were elevated. Quality of millipede compost has been compared with vermicompost and importance of pill millipedes in recycling plantation residues has been discussed.

Keywords: *Arthrosphaera magna*, compost, faecal pellets, nitrogen, organic matter.

EVEN though the global renewable lignocellulosic waste production per annum is about $20\text{--}50 \times 10^9$ tonnes, only about 4×10^9 tonnes is utilized¹. Decomposition and mineralization of such wastes are essential for continued soil productivity in terrestrial ecosystems². Decomposition decreases the mass of organic substance due to physical breakdown of substrate, leaching of soluble materials, and catabolism or oxidation³. Although composting has been considered as a means of disposal of waste, the resulting product has significant commercial value. Activities of a consortium of soil organisms bring about composting of plant residues, where saprophagous fauna plays a significant role. Composting organic wastes for agricultural use is one of the improved ways of organic farming. On-farm cycling of organic waste and application of compost are the most popular agronomic measures adopted to maintain soil quality and health. Several serious environmental perturbations can be resolved through management of decomposition or organic cycling⁴.

Vermicompost production through earthworms is one of the popular means of optimum utilization of organic

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