Lack of Association between IL-10 Gene Promoter Polymorphisms and Susceptible to Tuberculosis in Thai Patients

Manaphol Kulpraneet MD*, Anirut Limtrakul PhD**,
Surangrat Srisurapanon PhD***, Piyatida Tangteerawatana PhD**

** Department of Internal Medicine, Faculty of Medicine, Srinakharinwirot University, Nakhon Nayok, Thailand*
*** Department of Microbiology, Faculty of Medicine, Srinakharinwirot University, Bangkok, Thailand*
**** Department of Pathology, Faculty of Medicine, Srinakharinwirot University, Bangkok, Thailand*

Background: *Cytokines play a major role in defense against Mycobacterium tuberculosis infection. Polymorphisms in the genes encoding various cytokines have been associated with tuberculosis susceptibility. Polymorphisms of the regulatory cytokine gene, the interleukin (IL)-10 is associated with the risk of tuberculosis (TB) in different populations. However, IL-10 gene polymorphism and susceptibility to TB in Thai is still unknown.*
Objective: *The purpose of this study was to evaluate whether the common IL-10 promoter gene polymorphisms are associated with TB in Thai population.*
Material and Method: *Forty-eight patients with newly diagnosed pulmonary tuberculosis were studied. DNA samples were extracted from leukocytes and used to investigate -1087A/G, -819C/T, -252C/A (rs1800896, rs1800871, rs1800872) in IL-10 gene using restriction fragment length polymorphism (PCR-RFLP) methods.*
Results: *In this study, the genotype and allele frequencies of IL-10-1087A/G, -819C/T, -252C/A polymorphism did not significantly different between TB patients and healthy controls ((genotype: p = 0.38, p = 0.92, p = 1; allele: p = 0.57, p = 0.77, p = 0.89, respectively).*
Conclusion: *The lack of association between common IL-10 promoter polymorphisms and TB susceptibility in this study may provide clue for better understanding of IL-10-1087A/G, -819C/T, -252C/A polymorphism and TB susceptibility in Thai population, which might facilitate the rationale design of vaccines. However, further studies in large scales population are required for confirmation.*

Keywords: *IL-10, Cytokines, Single nucleotide polymorphism (SNP), Tuberculosis*

J Med Assoc Thai 2015; 98 (Suppl. 10): S124-S129
Full text. e-Journal: *http://www.jmatonline.com*

Tuberculosis (TB) remains a major global health problem. According to World Health Organization, about 32 percent of the world's population is infected with *Mycobacterium tuberculosis* (*M. tuberculosis*) with an estimated 8.6 million people developed TB and 1.3 million died from the disease annually. In Thailand, TB is still prevalent with approximately 65,000 cases every year. In 2012, Thailand ranked 18th among 22 countries with high burden of tuberculosis and the incidence rate was 119/100,000 population[1]. As only 10 percent of the population infected by *M. tuberculosis* will develop clinical TB, differences in host immunity and genetic factors may account for development after infection. The genetic influence on TB infection was established by many studies, indicating that genetics may play a role in the susceptibility to TB infection[2-4]. Cytokines play a main role in orchestrating the immune response which is activated as a network of pro-inflammatory and regulatory cytokines derived from both macrophages and T cells and determine the disease outcome in TB. Interleukin (IL)-10 is an important immunoregulatory cytokine mainly produced by macrophages, monocytes, T cells, B cells, dendritic cells, mast cells and eosinophils[5]. IL-10 downregulates the IFN-γ production of T cells, and the secretion of tumor

Correspondence to:
Tangteerawatana P, Department of Microbiology, Faculty of Medicine, Srinakharinwirot University, 114 Sukhumvit 23 Wattana, Bangkok 10110, Thailand.
Phone: +66-2-6495000 ext. 4946; Fax: +66-2-6495394
E-mail: piyatida@g.swu.ac.th

necrosis factor, nitric oxide and the expression of costimulatory molecules and MHC class II of macrophages[6]. Turner demonstrated that increased susceptibility to reactivation tuberculosis in the mouse model is strongly influenced by the expression of IL-10 during the chronic or latent phase of the infection[6]. IL-10 potentially helps *M. tuberculosis* persistence in humans by blocking phagosome maturation in macrophages[7]. The ability of IL-10 to down-regulate immune responses and the fact that IL-10 can be detected in tuberculosis patients have led researchers to investigate whether IL-10 plays a role in susceptibility to tuberculosis[8,9]. Gene encoding for IL-10 is located on human chromosome 1 (1q31-q32). Many single nucleotide polymorphisms (SNPs) were reported in the proximal (at position -1082A/G, (rs1800896), -819T/C (rs1800871), and -592A/C (rs1800872)) and distal regions of the IL-10 gene[10,11] and may directly affect cytokine production levels[11,12]. Previous study demonstrated that the -1082G/-819C/-592C (GCC), ACC and ATA haplotypes were associated with high, intermediate, and low IL-10 transcriptional activities, respectively[13]. Data on IL10 SNPs and susceptibility to TB are very inconsistent. Some studies in Korean, Colombian and Turkish populations found association of IL10 -1082A/G with susceptibility to TB, while other studies did not find any association for the IL10 -1082A/G SNP and TB[14-17]. The study in healthy Thai population, IL-10 -1082 A/G had two genotypes with frequency of 85% for AA and 15% for AG. IL-10-819C/T and IL-10-592C/A had three genotypes with similar frequency IL-10-819T/C; CC 12%, CT 43%, and TT 45% IL-10-592A/C CC 10%, CA 42%, and AA 48%. The IL-10 -1082A/G, IL-10 -819C/T and IL-10-592C/A genotype and allele frequencies in Thai are similar to Chinese and Japanese, but differ from Caucasian and African[18].

There were a number of case-control studies investigated IL-10 genetic variant in different populations toward susceptibility to TB, but the findings remain conflicting rather than conclusive. So far, lack of data on IL-10 promoter gene polymorphisms and susceptibility to TB in Thai population. Therefore, the aim of this work was to determine whether there is any association between IL-10 common single nucleotide gene polymorphisms and susceptibility to TB in Thai population.

Material and Method

Study groups

All patients were recruited from HRH princess Maha Chakri Sirindhorn Medical Center, Faculty of Medicine, Srinakharinwirot University, Ongkharak, Nakhon Nayok. These included 48 patients with newly diagnosed pulmonary TB. All patients were either smear/culture positive or with clinical-radiological and histological evidences for TB. Their mean age was 46.39 years for men and 45.95 for women, and 41.67% were women. According to patient history records, those patients had no sign of immunodeficiency and autoimmune diseases. Control group consisted of 100 adult healthy donors residing in Bangkok as previous report in Sodsai[18]. This study was approved by the Faculty of Medicine Srinakharinwirot University Ethical Review Committee (SWUEC/EX 23/2555) and all subjects gave written informed consent.

DNA purification

DNA was extracted from the buffy coat using a commercial extraction kit (Roche, Germany), according to the manufacturer's instructions and stored at minus 20°C until analysis.

PCR- RFLP analysis of the IL-10 polymorphisms

The IL-10 (-1087A/G rs1800896, -819C/T rs1800871, -592C/A rs1800872) was investigated using PCR-restriction fragment length polymorphism (RFLP) analysis as described previously[19,20]. Briefly, the PCR amplification was performed in 10 µl reactions mixture including 1 µl of genomic DNA, 5 µl of 2x ready-to-use PCR Master Mix (iNtRON biotechnology, Korea) and 5 pmoles of each SNP primer. The PCR was carried out in an Eppendorf (Corning, USA) using a 2 min denaturation at 94°C followed by 35 cycles with 94°C for 20 sec, 55°C for 10 sec and 72°C for 30 sec. The final extension was performed at 72°C for 5 min. The PCR products were then digested with specific restriction endonuclease EcoNI, MaeIII, or RsaI at 37°C for 1 hour. and analyzed on 2% agarose gel containing ethidium bromide. The following fragments were obtained: IL-10-1087: G/G 310 and 280 bp, A/G 310, 280, 252 and 28 bp, A/A 310, 252 and 28 bp; IL-10-819: TT 209 bp, TC 209, 125, and 84 bp, CC 125 , and 84 bp; IL-10-592: CC 412 bp. CA 412, 236 and 176 bp, AA 236 and 176 bp. The samples were test in duplicate by different persons, and the results were 100 percent concordant.

Statistical analysis

Allele and genotype frequencies were compared between patient and healthy control groups using the Chi-square (χ^2) test. A *p*-value of 0.05 or less was considered to be significant, and *p*-values between 0.05-0.1 were considered to be marginally significant.

Results

IL-10 (-1082 A/G, -819 C/T, -592 C/A) Genotype and allele frequencies

The genotype distribution of all 3 SNPs in IL-10 promoter was consistent with the assumption of Hardy-Weinberg equilibrium. The genotype and allele frequencies of the IL-10 promoter polymorphism are shown in Table 1. IL-10-1087 A/G had two genotypes: AA and AG with frequencies of 91.7% and 8.3%, respectively among TB patients and 85% and 15% respectively among control subjects[18]. IL-10-819 C/T had three genotypes: CC, CT, and TT with frequencies of 12.5%, 35.4%, and 52.1% respectively among TB patients and 12%, 43%, and 45% respectively among control subjects. Similar to IL-10-819 C/T, IL-10 -592 C/A had three genotypes: CC, CA, and AA with frequencies of 12.5%, 35.4%, and 52.1% respectively among TB patients, and 10%, 42%, and 48% respectively among control subjects. Allele -819T and -592A were seen together (69.8%) and were more frequent than -819C and -592C (30.2%). Thus, linkage disequilibrium was observed between allele -819T and -512A, which was similar to previous report where -819T and -592A together 71.9% and -819C and -592C together 28.1%[21]. TB patients and control group had very similar genotype and allele distributions among IL-10 -1082 G/A, -819 C/T, -592 C/A. No statistical significant difference for genotypes and allele frequency was observed between TB patients and control group (genotype: $p = 0.38$, $p = 0.92$, $p = 1$; allele:

Table 1. Analysis of IL-10 (-1087A/G rs1800896, -819 C/T rs1800871, -592C/A rs1800872) genotype and allele frequencies in Tuberculosis patients

	Healthy controls (a)	TB patients (b)		
SNP designation	n = 100	n = 48 (%)	*p*-value (c)	HWE (d)
1087 A/G				
Genotype				
AA	85	44 (91.7)	0.38	0.47
AG	15	4 (8.3)		
GG	0	0		
Allele				
A	93	95.8	0.57	
G	7	4.2		
819 C/T				
Genotype				
CC	12	6 (12.5)	0. 92	0.64
CT	43	17 (35.4)		
TT	45	25 (52.1)		
Allele				
C	33	30.2	0.77	
T	67	69.8		
592 C/A				
Genotype				
CC	10	6 (12.5)	1	0.64
CA	42	17 (35.4)		
AA	48	25 (52.1)		
Allele				
C	32	30.2	0.89	
A	68	69.8		

(A) Data for IL-10 (-1082A/G rs1800896, -819C/T rs1800871, -592C/A rs1800872) genotype and allele frequencies of healthy controls were obtained from Ref 18. (B) Data for IL-10 (-1087A/G rs1800896, -819C/T rs1800871, -592C/A rs1800872) genotype and allele frequencies of TB patients were obtained from present study. (C) Chi-square test was used for the analysis. (D) Hardy-Weinberg equilibrium in TB patients.
IL-10-1082A/G and IL-10-1087A/G are the same SNP

$p = 0.57$, $p = 0.77$, $p = 0.89$, respectively).

Discussion

In this study, the common IL-10 promoter gene polymorphisms were investigated in newly diagnosed pulmonary TB patients in Thai population. IL-10 -1087A/G, -819T/C and -592A/C polymorphisms did not show significant association with pulmonary TB susceptibility.

IL-10 is a powerful regulatory cytokine and plays an essential role during the latent TB stage, where increased production of this cytokine promotes reactivation of disease in mice[6] and suppression of cell-mediated immunity against the intracellular infection[3]. IL-10 gene is located on the long arm of chromosome 1, where several polymorphisms have been identified within the promoter region, such as -1082A/G, -819C/T, and -592C/A. It has been reports that genotype frequencies at IL-10 vary greatly in different populations, particularly in individuals of different ethnicities[22]. The allele frequencies of IL-10 -1087A, -819T, -592A in Thai TB patients in present study (95.8%, 69.8%, 69.8%) was similar to those reports in healthy population in Thai (97%, 67%, 68%), Chinese (94%, 69%, 70%) and Japanese (97%, 67%, 68%)[18]. When compare the percent frequencies of IL-10-1087A/G, -819C/T, -592C/A in TB patients in present study with the previous reports in Thai healthy control[18], no statistically significant difference in IL-10 -1087A/G, -819C/T, -592C/A genotype and allele frequency between the two group were seen. This may suggest that IL-10 common polymorphisms did not show significant association with TB susceptibility in Thai population. This result inconsistent with the recent meta-analysis report that the IL-10-819C/T and -592C/A polymorphisms were significantly associated with TB risk in Asians, but consistent with those report that the IL-10 -1082A/G genotype were not associated with TB risk in Asians or Africans[23].

In conclusion, in Thai pulmonary TB patients the disease susceptibility would not be influenced by IL-10 -1087G/A, -819C/T and -592C/A polymorphisms. However, this study may facilitate the rational design of vaccines and/or therapeutics. Additional studies in large scales population are required for confirmation.

What is already known on this topic ?

A number of case-control studies investigated IL-10 genetic variant in different populations toward susceptibility to TB, but the findings remain conflicting rather than conclusive. Recently, a meta analysis reviewed that IL-10-1082G/A associated with TB in European, and IL-10 -819C/T and -592C/A associated with TB in Asian population.

What this study adds ?

So far, lack of data on IL-10 promoter gene polymorphisms and susceptibility to TB in Thai population. This study demonstrated that in newly diagnosed Thai pulmonary TB patients, the disease susceptibility would not be influenced by IL-10 -1087G/A, 819C/T and -592C/A polymorphisms.

Acknowledgement

We sincerely thank all patients for participating in the study. This work was supported by the Grant of Faculty of Medicine, HRH princess Maha Chakri Sirindhorn Medical Center, Srinakharinwirot University (grant reference number 199/2555 to PT).

Potential conflicts of interest

None.

References

1. World Health Organization. 2012 Report on Tuberculosis Control in the South-East Asia region. [Internet] WHO; 2012 [cited 2015 Dec 16]. Available from: http://whothailand.healthrepository.org/handle/123456789/1430
2. Hill AV. The genomics and genetics of human infectious disease susceptibility. Annu Rev Genomics Hum Genet 2001; 2: 373-400.
3. Moller M, Hoal EG. Current findings, challenges and novel approaches in human genetic susceptibility to tuberculosis. Tuberculosis (Edinb) 2010; 90: 71-83.
4. Vannberg FO, Chapman SJ, Hill AV. Human genetic susceptibility to intracellular pathogens. Immunol Rev 2011; 240: 105-16.
5. Ouyang W, Rutz S, Crellin NK, Valdez PA, Hymowitz SG. Regulation and functions of the IL-10 family of cytokines in inflammation and disease. Annu Rev Immunol 2011; 29: 71-109.
6. Turner J, Gonzalez-Juarrero M, Ellis DL, Basaraba RJ, Kipnis A, Orme IM, et al. In vivo IL-10 production reactivates chronic pulmonary tuberculosis in C57BL/6 mice. J Immunol 2002; 169: 6343-51.
7. O'Leary S, O'Sullivan MP, Keane J. IL-10 blocks phagosome maturation in mycobacterium tuberculosis-infected human macrophages. Am J Respir Cell Mol Biol 2011; 45: 172-80.

8. Barnes PF, Lu S, Abrams JS, Wang E, Yamamura M, Modlin RL. Cytokine production at the site of disease in human tuberculosis. Infect Immun 1993; 61: 3482-9.
9. Verbon A, Juffermans N, Van Deventer SJ, Speelman P, Van Deutekom H, Van Der PT. Serum concentrations of cytokines in patients with active tuberculosis (TB) and after treatment. Clin Exp Immunol 1999; 115: 110-3.
10. D'Alfonso S, Rampi M, Rolando V, Giordano M, Momigliano-Richiardi P. New polymorphisms in the IL-10 promoter region. Genes Immun 2000; 1: 231-3.
11. Mormann M, Rieth H, Hua TD, Assohou C, Roupelieva M, Hu SL, et al. Mosaics of gene variations in the Interleukin-10 gene promoter affect interleukin-10 production depending on the stimulation used. Genes Immun 2004; 5: 246-55.
12. Eskdale J, Gallagher G, Verweij CL, Keijsers V, Westendorp RG, Huizinga TW. Interleukin 10 secretion in relation to human IL-10 locus haplotypes. Proc Natl Acad Sci U S A 1998; 95: 9465-70.
13. Turner DM, Williams DM, Sankaran D, Lazarus M, Sinnott PJ, Hutchinson IV. An investigation of polymorphism in the interleukin-10 gene promoter. Eur J Immunogenet 1997; 24: 1-8.
14. Oh JH, Yang CS, Noh YK, Kweon YM, Jung SS, Son JW, et al. Polymorphisms of interleukin-10 and tumour necrosis factor-alpha genes are associated with newly diagnosed and recurrent pulmonary tuberculosis. Respirology 2007; 12: 594-8.
15. Henao MI, Montes C, Paris SC, Garcia LF. Cytokine gene polymorphisms in Colombian patients with different clinical presentations of tuberculosis. Tuberculosis (Edinb) 2006; 86: 11-9.
16. Ben Selma W, Harizi H, Boukadida J. Association of TNF-alpha and IL-10 polymorphisms with tuberculosis in Tunisian populations. Microbes Infect 2011; 13: 837-43.
17. Lopez-Maderuelo D, Arnalich F, Serantes R, Gonzalez A, Codoceo R, Madero R, et al. Interferon-gamma and interleukin-10 gene polymorphisms in pulmonary tuberculosis. Am J Respir Crit Care Med 2003; 167: 970-5.
18. Sodsai P, Nakkuntod J, Kupatawintu P, Hirankarn N. Distribution of cytokine gene polymorphisms in Thai population. Tissue Antigens 2011; 77: 593-7.
19. Padyukov L, Hahn-Zoric M, Lau YL, Hanson LA. Different allelic frequencies of several cytokine genes in Hong Kong Chinese and Swedish Caucasians. Genes Immun 2001; 2: 280-3.
20. Shih CM, Lee YL, Chiou HL, Hsu WF, Chen WE, Chou MC, et al. The involvement of genetic polymorphism of IL-10 promoter in non-small cell lung cancer. Lung Cancer 2005; 50: 291-7.
21. Netsawang J, Tangwattanachuleeporn M, Hirankarn N, Wongpiyabovorn J. The distribution of IL-10 promoter polymorphism in Thais. J Med Assoc Thai 2004; 87 (Suppl 2): S117-22.
22. Meenagh A, Williams F, Ross OA, Patterson C, Gorodezky C, Hammond M, et al. Frequency of cytokine polymorphisms in populations from western Europe, Africa, Asia, the Middle East and South America. Hum Immunol 2002; 63: 1055-61.
23. Liang B, Guo Y, Li Y, Kong H. Association between IL-10 gene polymorphisms and susceptibility of tuberculosis: evidence based on a meta-analysis. PLoS One 2014; 9: e88448.

การศึกษาความสัมพันธ์ระหว่างความหลากหลายของจีน IL-10 กับโอกาสการเกิดโรควัณโรคในกลุ่มคนไทย

มนะพล กุลปราณีต, อนิรุทธิ์ ลิ้มตระกูล, สุรางค์รัตน์ ศรีสุรภานนท์, ปิยะธิดา ตั้งธีระวัฒนะ

ภูมิหลัง: cytokine มีบทบาทสำคัญในกลไกการเกิดโรควัณโรค IL-10 เป็น cytokine หนึ่งที่ควบคุมการทำงานของระบบภูมิคุ้มกัน ความหลากหลายของจีน IL-10 มีความสัมพันธ์กับโอกาสการเกิดโรคและความรุนแรงของโรควัณโรคในหลายเชื้อชาติ อย่างไรก็ตามยังไม่พบรายงานการศึกษาความหลากหลายของจีน IL-10 กับโอกาสเกิดโรควัณโรคในคนไทย

วัตถุประสงค์: เพื่อศึกษาความหลากหลายของจีน IL-10 กับความเสี่ยงในการเป็นโรควัณโรคในกลุ่มคนไทย

วัสดุและวิธีการ: โดยศึกษา single neucleotide polymorphisms ของ promoter ของจีน IL-10 ตำแหน่ง IL-10-1087A/G, -819C/T, -252C/A ในผู้ป่วยวัณโรคปอด 48 คน ด้วยวิธี restriction fragment length polymorphism (PCR-RFLP)

ผลการศึกษา: ความถี่ของ genotype และ allele IL-10-1087A/G, -819C/T, -252C/A ในผู้ป่วยวัณโรคปอดและคนปกติไม่มีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติ

สรุป: จากการศึกษาครั้งนี้ไม่พบความสัมพันธ์ระหว่างความหลากหลายของจีน IL-10-1087A/G, -819C/T, -252C/A กับโอกาสเกิดโรควัณโรค อย่างไรก็ตามข้อมูลที่ได้น่าจะนำไปใช้ในการออกแบบวัคซีนและ/หรือการรักษาโรคได้ในอนาคต