

Marginal Evidence on an Association Between Interleukin-10 Production and Polymorphisms of the *IL10* Gene in Former Bacillus Calmette-Guerin Osteitis Patients

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Abstract

Introduction: Interleukin-10 (IL-10) production encoded by the *IL10* gene has been associated with the tuberculosis (TB) risk. Bacillus Calmette-Guerin (BCG) vaccination against TB may cause complications like osteitis. Our aim was to evaluate the association between four single nucleotide polymorphisms (SNP) of the *IL10* gene and serum IL-10 concentrations in former BCG osteitis patients.

Methods: Serum IL-10 concentrations measured in samples from 130 adults with BCG osteitis in infancy were compared with regard to the genotypes and haplotypes of *IL10* rs1800896 (-1082 A/G), rs1800871 (-819 C/T), rs1800872 (-592 C/A) and rs1800890 (-3575 A/T) SNPs.

Results: The variant genotypes of *IL10* rs1800871 (CT or TT) and rs1800872 (CA or AA) SNPs were marginally associated with lower serum IL-10 concentrations ($p = 0.06$). Serum IL-10 was 8.94 pg/mL if the haplotype ATA of *IL10* rs1800896, rs1800871 and 1800872 was present and 12.36 pg/mL if the haplotype ATA was absent ($p = 0.08$).

Conclusion: There were no significant associations between the four studied *IL10* gene promoter region polymorphisms and serum IL-10 levels. However, both genotype and haplotype analyses suggested that the variant rs1800871 and rs1800872 SNPs may be associated with low IL-10 production.

Keywords: *Bacillus Calmette-Guerin*; BCG Vaccination; BCG Osteitis; Interleukin-10; Interleukin10 Gene; Single Nucleotide Polymorphism; Tuberculosis; bacteriophage

Abbreviations

BCG: Bacillus Calmette-Guerin; IL-10: Interleukin-10 (Protein); *IL10*: *Interleukin10* (Gene); SNP: Single Nucleotide Polymorphism; TB: Tuberculosis.

Introduction

Interleukin-10 (IL-10) is one of the most important anti-inflammatory cytokines, mainly preventing and reducing immune reactions and tissue damage [1]. The ability to produce IL-10 varies between individuals due to genetically determined differences [2,3].

In previous studies, IL-10 has been involved with many diseases, including tuberculosis (TB) [4]. The impact of different *IL10* gene single nucleotide polymorphisms (SNPs) has varied depending on the population studied. In systematic reviews with meta-analyses, the SNP rs1800896 (-1082 A/G) was a risk factor for TB in Europeans [5-7] and North Americans [6] and the SNPs rs1800871 (-819 T/C) and rs1800872 (-592 A/C) in Asians [6,7].

The *IL10* gene is highly polymorphic and point mutations in the proximal promoter region including rs1800896, rs1800071 and rs1800872 form distinct haplotypes that are associated with IL-10 production [8]. High, intermediate and low IL-10 production has been associated with the haplotypes GCC, ACC and ATA, respectively [8,9].

Although *IL10* gene polymorphisms have been associated with an increased susceptibility to TB [5,6] the associations with other mycobacteria like Bacillus Calmette-Guerin (BCG) are not known [11].

In our study published in 2015 [12], there were no significant differences between 132 former BCG osteitis patients and population controls with regard to the SNPs of the *IL10* genes rs1800896 (-1082 A/G), rs1800871 (-819 C/T), rs1800872 (-592 C/A) or rs1800890 (-3575 T/A), or any of the rs1800896, rs1800071 and rs1800872 haplotypes. The aim of the present study was to evaluate whether there are associations between these four polymorphisms and serum IL-10 concentrations in the same cohort.

Methods

In Finland, 98% of newborn infants received BCG vaccination from 1951 until 2006, when universal BCG vaccinations were discontinued and vaccinations were limited to certain risk groups [13]. The microbe-specific diagnostics of invasive BCG infections was centralized in one laboratory at the National Public Health Institute, Helsinki, from 1960 to 1988 and 222 BCG osteitis cases were diagnosed during that period [14,15]. In 2007 - 2008 we invited 203 former BCG osteitis patients to take part in this study and 160 (78.8%) returned the questionnaire and 132 (65%) gave blood samples. Acute diseases or exacerbations of chronic diseases were not allowed at the time of sampling [12]. Fresh blood samples were collected into heparinized tubes and transported within 12 hours to the Tuberculosis Reference Laboratory at the National Institute for Health and Welfare, Turku, Finland. When the samples arrived, DNA was isolated and serum was separated and frozen at -70°C [16].

The genetic methods have been published previously, together with the results of the *IL10* rs1800896, rs1800871, rs1800872 and rs1800890 SNPs for 132 former BCG osteitis patients and about 400 controls [12]. Control samples were obtained from a prospective birth cohort study (STEPS) at the age of 2 - 3 months [12,17]. There were no significant differences between cases and controls with regard to the genotypes or minor or major allele frequencies of the *IL10* rs1800896, rs1800871, rs1800872 or rs1800890 SNPs [12]. Likewise, there were no significant differences between the cases and controls with regard to the haplotypes of the *IL10* rs1800896, rs1800871 and rs1800872 SNPs [12].

Laboratory analyses

The Bio-Plex Pro human IL-10 immunoassay kit (Bio-Rad, Helsinki, Finland) was used to measure serum IL-10 in 130/132 frozen serum samples, using the Bio-Plex 200 System (Bio-Rad, Helsinki, Finland) according to the manufacturer's protocol. The detection limit was 2 pg/mL. One sample with a known IL-10 concentration was used as an internal control for all the runs.

First, the serum concentrations of IL-10 were compared in relation to the genotypes of the *IL10* rs1800896 (-1082 A/G), rs1800871 (-819 C/T), rs1800872 (-592 C/A) and rs1800890 (-3575 A/T). Second, the concentrations were compared in relation to the haplotypes of *IL10* rs1800896, rs1800071 and rs1800872, which are situated at the proximal promoter region of the gene [18]. Since the *IL10* rs1800071 and rs1800872 SNPs are in full linkage, the possible haplotypes of the *IL10* gene rs1800896 (-1082 A/G), rs1800871 (-819 C/T) and rs1800872 (-592 C/A) were ACC, ATA, GCC and GTA, respectively [19].

Ethics

The study was approved by the Ethics Committee of Tampere University Hospital Districts, Tampere, Finland. The study patients were approached by postal mail and volunteers provided written, informed consent for further genetic studies on susceptibility to BCG osteitis. The studies in the genetics laboratory were carried out as coded without any personal data.

Statistics

The Statistical Package for the Social Sciences, Windows version 19.0 (IBM Corp, Armonk, NY, USA) was used for statistical analyses. Exploratory data analyses revealed that serum IL-10 concentrations were non-normally distributed. Therefore, the Mann-Whitney U test was used to compare IL-10 concentrations between the carriers of the genotypes and the haplotypes. The results are given as medians, 25% to 75% interquartile (IQ) ranges and minimum to maximum ranges. The heterozygous, homozygous and combined (homozygous plus heterozygous) variants were separately compared with the wild genotypes. The haplotype carriers were compared with those who did not have that specific haplotype.

Results and Discussion

The median concentration of serum IL-10 at the age of 21 - 49 years was 9.94 pg/mL (IQ 6.67-27.38pg/mL, range 0.00-5877.96 pg/mL) in the 130 frozen serum samples of the study subjects with BCG osteitis after newborn BCG vaccination. The median concentration was 9.88 pg/mL (IQ 6.44 - 32.04pg/mL) in the 59 males and 10.00 pg/mL (IQ 7.00 - 26.36 pg/mL) in the 71 females. The median concentration by age group was 17.92 pg/mL (IQ 9.74 - 30.44 pg/mL) in those 21 aged 21 to 30 years, 9.30 pg/mL (IQ 6.45 - 22.27 pg/mL) in those 88 aged 31 to 40 years and 12.40 pg/mL (IQ 5.88 - 58.98 pg/mL) in those 21 aged 41 to 49 years.

As seen in Table 1, there were no significant differences in serum IL-10 concentrations between study subjects with wild or variant genotypes of the *IL10* rs1800896 (-1082 A/G), rs1800871 (-819 C/T), rs1800872 (-592 C/A) or rs1800890 (-3575 A/T) SNPs. The heterozygous variant genotypes of the *IL10* rs1800871 (CT) and rs1800872 (CA) SNPs were associated with significantly lower serum IL-10 concentrations ($p = 0.03$) than those with the wild (CC) genotype. However, the respective differences were only marginal when the combined variant heterozygous and homozygous genotypes were compared with the wild genotype ($p = 0.06$).

Genotypes	Serum IL-10 concentration (pg/mL) Median (IQ) [Range]	P-value versus wild genotype
<i>IL10</i> rs1800896 AA, wild (n=43)	10.56 (7.72-33.00) [0.00-1379.48]	---
<i>IL10</i> rs1800896 AG, variant (n=54)	9.48 (5.75-21.57) [1.00-356.84]	0.46
<i>IL10</i> rs1800896 GG, variant (n=28)	10.70 (6.61-38.72) [2.12-5877.96]	0.85
<i>IL10</i> rs1800896 AG or GG, variant (n=82)	9.80 (6.39-23.69) [1.00-5877.96]	0.66
<i>IL10</i> rs1800871 CC and rs1800872 CC, wild (n=78)	12.38 (6.91-38.69) [1.36-5877.96]	---
<i>IL10</i> rs1800871 CT and rs1800872 CA, variant (n=41)	8.88 (5.52-15.50) [0.00-1379.48]	0.03
<i>IL10</i> rs1800871 TT and rs1800872 AA, variant (n=8)	9.54 (8.22-51.74) [5.56-64.08]	0.94
<i>IL10</i> rs1800871 CT or TT and rs1800872 CA or AA, variant (n=49)	8.88 (6.16-15.72) [0.00-1379.48]	0.06
<i>IL10</i> rs1800890 AA, wild (n=54)	11.34 (7.54-32.43) [0.00-1379.48]	---
<i>IL10</i> rs1800890 AT, variant (n=55)	8.84 (5.72-18.32) [1.00-356.84]	0.10
<i>IL10</i> rs1800890 TT, variant (n=21)	17.08 (9.62-64.68) [3.04-5877.96]	0.12
<i>IL10</i> rs1800890 AT or TT, variant (n=76)	9.80 (6.30-25.37) [1.00-5877.96]	0.52

Table 1: Concentrations of serum IL-10 in relation to the genotypes of the *IL10* gene SNPs rs1800896 (-1082 A/G), rs1800871 (-819 C/T), rs1800872 (-592 C/A), and rs1800890 (-3575 A/T) in 130 former BCG osteitis patients.

IL10 rs1800871 and *IL10* rs1800872 were in full linkage: allele C at rs1800871 was always linked with allele C at rs1800872 and allele T at rs1800871 was always linked with allele A at rs1800872.

In previous studies, high production of the anti-inflammatory cytokine IL-10 has been related to poor outcome of mycobacterial infections [20,21]. In a computerized model, low IL-10 production was associated with an early recovery from TB [22]. In experimental studies in mice, blocking of IL-10 receptor signalling during BCG vaccination increased protection against *Mycobacterium tuberculosis* [23] and treatment with anti-IL-10 receptors during *M. tuberculosis* infection resulted in long-term control of the disease [24].

There were no significant differences in serum IL-10 concentrations between the subjects with the haplotypes GCC, GTA, ACC or ATA of rs1800896 (-1082 A/G), rs1800871 (-819 C/T) or rs1800872 (-592 C/A) and those without the respective haplotypes (Table 2). However, serum IL-10 was 8.94 pg/mL if the haplotype ATA was present and 12.36 pg/mL if the haplotype ATA was absent (p = 0.08) in the current study (Table 2).

In previous studies, high, intermediate and low IL-10 production has been associated with the *IL10* gene rs1800896 (A/G), rs1800871 (T/C) and rs1800872 (A/C) haplotypes of GCC (high), ACC (intermediate) and ATA (low), respectively [8-10]. The ATA haplotype consists of the wild-type allele A in the *IL10* rs1800896 and of variant-type alleles T and A in the *IL10* rs1800871 and 1800872, respectively.

Carriers of allele combinations (haplotype)	Serum IL-10 concentration (pg/mL) in presence of the haplotypes Median (IQ) [Range]	Serum IL-10 concentration (pg/mL) in absence of the haplotype Median (IQ) [Range]	P-value versus those without the haplotype
GCC [G ¹⁸⁰⁰⁸⁹⁶ , C ¹⁸⁰⁰⁸⁷¹ , C ¹⁸⁰⁰⁸⁷²] (n = 82)	9.80 (6.39-23.69) [1.00-5877.96]	10.56 (7.72-33.00) [0.00-1379.48]	0.66
GTA [G ¹⁸⁰⁰⁸⁹⁶ , T ¹⁸⁰⁰⁸⁷¹ , A ¹⁸⁰⁰⁸⁷²] (n = 21)	9.24 (4.74-15.50) [1.00-93.44]	9.92 (4.74-15.50) [1.00-93.44]	0.19
ACC [A ¹⁸⁰⁰⁸⁹⁶ , C ¹⁸⁰⁰⁸⁷¹ , C ¹⁸⁰⁰⁸⁷²] (n = 89)	9.72 (6.52-24.46) [0.00-1379.48]	10.20 (7.09-38.72) [2.12-5877.96]	0.44
ATA [A ¹⁸⁰⁰⁸⁹⁶ , T ¹⁸⁰⁰⁸⁷¹ , A ¹⁸⁰⁰⁸⁷²] (n = 48)	8.94 (5.94-15.82) [0.00-1379.48]	12.36 (6.82-38.90) [1.36-5877.96]	0.08

Table 2: Concentrations of serum IL-10 in relation to the potential carriers of the haplotypes of *IL10* gene SNPs rs1800871 (-819 C/T), rs1800872 (-592 C/A), and rs1800896 (-1082 A/G) in 130 former BCG osteitis patients.

IL10 rs1800871 and *IL10* rs1800872 were in full linkage: therefore, only haplotypes GCC, GTA, ACC and ATA were possible.

Although the number of BCG osteitis patients was higher than in any other study and all belonged to the homogenous Finnish population, the sample size was small meaning a risk of under-powering in analyses stratified by genetic findings. There are three other obvious limitations in this study. First, IL-10 production is highly variable over time and the samples were obtained years after BCG osteitis. Second, the study was not controlled in terms of serum IL-10 concentrations. Third, our way to construct the haplotypes recognized only potential carriers. The models that take into account the frequencies of alleles in the population in question, give frequencies that are exact and specific in the studied population, but they do not identify individual cases [25].

Conclusion

The mainly negative results of this study suggest that the four *IL10* promoter-region polymorphisms we studied, were not involved in IL-10 production in former BCG osteitis patients. However, minor non-significant evidence was found that the variant genotypes of the rs1800871 and rs1800872 and the ATA haplotype consisting of the wild-type A allele at rs1800896 and the variant-type T and A alleles at rs1800871 and rs1800872, respectively, were associated with low serum IL-10 concentrations. Recently, the *IL10* rs1518111 polymorphism, which we did not study, increased the susceptibility to non-tuberculosis mycobacterial infections [26].

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Conflicts of Interest

The authors declare no conflicts of interest.

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