ECRONICON OPEN ACCESS

EC MICROBIOLOGY
Research Article

Serum Interferon-γ and Interleukin-12 in Relation to *IFNγ, IL12A* and *IL12B* Gene Polymorphisms in BCG Osteitis Survivors

Matti Korppi[1]*, Johanna Teräsjärvi[2], Eero Lauhkonen[1], Heini Huhtala[3] and Qiushui He[2,4]

[1]*Center for Child Health Research, Faculty of Medicine and Biotechnology, University of Tampere and University Hospital, Tampere, Finland*

[2]*Institute of Biomedicine, University of Turku, Turku, Finland*

[3]*Faculty of Social Sciences, University of Tampere, Tampere, Finland*

[4]*Department of Medical Microbiology, Capital Medical University, Beijing, China*

***Corresponding Author:** Matti Korppi, Professor, Center for Child Health Research, Faculty of Medicine and Biotechnology, University of Tampere and University Hospital, Tampere, Finland.

Received: November 21, 2019; **Published:** February 27, 2020

Abstract

Background: Interferon-γ (IFN-γ) and interleukin-12 (IL-12) are involved in responses to mycobacteria including BCG strains in the BCG vaccine. The aim of the study was to evaluate IFN-γ and IL-12 concentrations in serum in relation to two *IFNγ*, two *IL12A* and one *IL12B* polymorphisms, and to compare serum IFN-γ and IL-12 concentrations with *ex vivo* IFN-γ or IL-12 production in BCG-stimulated cell cultures.

Methods: Serum samples were obtained from 132 adults with BCG osteitis in infancy and good-quality samples were available for IFN-γ (N = 128) and IL-12p70 (N = 130) measurements using the Bio-Plex Pro human IL 10 immunoassay kit with the Bio-Plex 200 System. Serum IFN-γ and IL-12p70 concentrations were compared in relation to the *IFNγ* rs2430561, *IFNγ* rs35314021, *IL12A* rs568408, *IL12A* rs2243115 and *IL12B* rs3212227 genotypes, as well as in relation to *ex vivo* IFN-γ and IL-12p70 concentrations in BCG-stimulated cell cultures.

Results: There was no significant association between the five included *IFNγ*, *IL12A* or *IL12B* genotypes and serum IFN-γ or IL-12p70 concentrations. The result remained negative, when serum IFN-γ and IL-12p70 concentrations were compared between ten *ex vivo* low producers and those 104 who were not, respectively.

Discussion: The *IFNγ* rs2430561, *IFNγ* rs35314021, *IL12A* rs568408, *IL12A* rs2243115 and *IL12B* rs3212227 polymorphisms had no impact on *IFN-γ* and IL-12 production. Since IFN-γ and IL-12 are no doubt involved in responses to mycobacteria BCG included, other *IFNγ* and *IL12* polymorphisms need to be included in future studies.

Keywords: *Bacillus Calmette-Guerin; BCG osteitis; Gene Polymorphism; Interferon-γ; Interleukin-12; IFNγ Gene; IL12A Gene; IL12B Gene*

Abbreviations

BCG: Bacillus Calmette-Guerin; IFN-γ: Interferon-γ; IL-12: Interleukin-12; IFN-γ: Interferon-γ

Introduction

Finnish newborns were vaccinated as part of the general national vaccination program with the Bacillus Calmette-Guerin (BCG) vaccine from the 1940`s until 2006 and 222 children presented with BCG osteitis from 1960 to 1988 [1,2]. In 2007 - 2008, 160 previous

BCG osteitis patients attended the questionnaire study at 21 - 49 years of age, and blood samples were obtained for immunological and genetic studies from 132 study subjects [3].

Interferon-γ (IFN-γ) and interleukin-12 (IL-12) are involved, partly through the same pathway, in responses to mycobacteria including BCG strains of the BCG vaccine [4]. The production of IFN-γ and IL-12 cytokines, and the structures of the molecules in question are encoded by *IFNγ*, *IL12A* and *IL12B* genes, respectively. IL-12 consists of two particles, called as p35 and p40, and the *IL12A* gene encodes IL-12p35 and *IL12B* gene IL-12p40. IL-12 can act either as a homodimer (IL-12p40) or as a heterodimer (IL-12p70).

Recently, we published our results on *IFNγ* rs2430561, *IFNγ* rs35314021, *IL12A* rs568408, *IL12A* rs2243115 and *IL12B* rs3212227 genotypes in 132 former BCG osteitis patients [5]. The distributions of these five genotypes did not differ between BCG osteitis cases and population controls of the FIN data of the 1000 Genomes Project [6]. In supplementary analyses, the minor allele frequencies of the *IFNγ* rs2430561, *IFNγ* rs35314021 and *IL12A* rs2243115 did not differ between cases and 3472 Finnish controls from the publicly available genome aggregation database (https://gnomad.broadinstitute.org), but the *IL12A* rs568408 and *IL12B* rs3212227 differed. The presence of the wild versus variant genotype of these two *IFNγ* and three *IL12A* or *IL12B* polymorphisms had no significant associations with *ex vivo* production of IFN-γ and IL-12p70, respectively, in BCG-stimulated cell cultures [5].

Aims of the Study

The aim of the present study as to evaluate IFN-γ and IL-12 concentrations in serum in relation to the presence the *IFNγ* rs2430561, *IFNγ* rs35314021, *IL12A* rs568408, *IL12A* rs2243115 or *IL12B* rs3212227 genotypes, and to compare serum IFN-γ and IL-12 concentrations between those who were low *ex vivo* producers of IFN-γ or IL-12, respectively, in BCG-stimulated cell cultures and those who were not.

Materials and Methods

Originally, 222 infants presented with BCG osteitis from 1960 to 1988 in Finland, and 132 former BCG osteitis patients gave blood samples in 2007 - 2008 when they were 21 - 49 years old adults. The samples were frozen and stored in the National Public Health Institute, Turku, Finland, which was at that time the national tuberculosis reference laboratory, and later transferred for further immunological and genetic studies to the laboratory of Medical Microbiology and Immunology, University of Turku, Turku, Finland.

Good-quality serum samples obtained and frozen at -70°C in 2007 - 2008, were available for IFN-γ measurement in 128 cases and for IL-12p70 measurement in 130 cases. The Bio-Plex Pro human IL 10 immunoassay kit with the Bio-Plex 200 System (Bio-Rad, Helsinki, Finland) was used to measure IFN-γ and IL-12p70 cytokine concentrations [7,8]. The detection limit was 2.0 pg/ml for both IFN-γ and IL-12p70. Serum IFN-γ and IL-12p70 concentrations were compared in relation to the previously determined *IFNγ* rs2430561, *IFNγ* rs35314021, *IL12A* rs568408, *IL12A* rs2243115 and *IL12B* rs3212227 genotypes, respectively.

In addition, serum IFN-γ and IL-12 concentrations were compared between those 10 who were low *ex vivo* producers of IFN-γ and/or IL-12 and those 104 who were not. As described previously [3], low *ex vivo* producers were identified based on IFN-γ and/or IL-12 production in cell cultures stimulated with BCG and IL-12 when IFN-γ was measured, and with BCG and IFN-γ when IL-12 was measured, respectively.

Statistics

Statistical analyses were performed using the Statistics Package for Social Sciences (SPSS 25.0, IBM Corp., NY, USA). Since serum IFN-γ and IL-12 concentrations were non-normally distributed, the Mann-Whitney U test was used to compare the concentrations between the *IFNγ* or *IL12* wild versus variant genotypes, and between the low *ex vivo* IFN-γ or IL-12 producers and those who were not.

Ethics

The study was approved by the Ethics Committee of the Tampere University Hospital district. The study subjects gave their voluntary, informed, written consent for studies including permission to perform immunological and genetic analyses concerning susceptibility to BCG vaccination complications.

Results

There were no significant associations between the *IFNγ* rs2430561, *IFNγ* rs35314021, *IL12A* rs568408, *IL12A* rs2243115 or *IL12B* rs3212227 genotypes and serum IFN-γ or IL-12p70 concentrations, respectively (Table 1).

Genotypes	Median (pg/mL)	25th - 75th percentile (Q1-Q3)	P value (vs. wild)
***IFNγ* rs2430561#**			
Wild TT (N = 56)	37.8	3.5-69.4	---
Variant AT (N = 50)	19.2	8.6-47.5	0.870
Variant AA (N = 24)	15.2	7.0-35.1	0.304
Variant AT or AA (N = 74)	28.2	0-116.5	0.730
***IL12A* rs568408**			
Wild GG (N = 82)	19.2	8.6-47.5	---
Variant AG or AA (N = 48)	15.2	7.0-35.1	0.265
***IL12A* rs2243115**			
Wild TT (N = 103)	16.8	7.9-47.2	---
Variant GT or TT (N = 27)	15.2	7.9-44.6	0.934
***IL12B* rs3212227**			
Wild TT (N = 93)	15.2	7.6-43.9	---
Variant GT or GG (N = 37)	22.2	10.0-47.7	0.419

Table 1: *Serum IFN-γ and IL-12 concentrations in relation to the IFNγ rs2430561 and IL12A rs568408, IL12A rs2243115 and IL12B rs3212227 genotypes.*

#: The results concern also the wild and variant genotypes of the IFNγ rs35314021, since the IFNγ rs2430561 and rs35314021 show a 100% identity in the Finnish population data. As published previously [5], this co-segregation was in this material confirmed by sequencing 25 random samples.

The result remained negative, when serum IFN-γ or IL-12p70 concentrations were compared between the 10 *ex vivo* low producers and those 104 who were not, respectively. Median IFN-γ in serum was 66.7 pg/ml (25 - 75% range 21.2 - 137.0) vs. 34.1 (6.7 - 104.6, p = 0.077), and median IL-12 was 20.3 pg/ml (5.2 - 34.8) vs. 16.3 (7.9 - 44.6, p = 0.702).

Discussion

The results of the present study remained negative. We were not able to find any significant associations between the two included *IFNγ* genotypes, two included *IL12A* genotypes or one included *IL12B* genotype and serum IFN-γ or IL-12 concentrations, respectively. Likewise, serum IFN-γ and IL-12 concentrations did not differ between those who were low *ex vivo* producers of IFN-γ or IL-12 in stimulated cell cultures and those who were not. The median serum IFN-γ concentration was two-fold higher in low *ex vivo* IFN-γ producers than in those who were not. Statistically, the significance of the result was suggestive, but clinically, the direction of the difference was against our observations.

As discussed previously in details [5], the functionalities of the *IFNγ* rs2430561, *IFNγ* rs35314021 and *IL12B* rs3212227 have been well documented, but corresponding functionality data are lacking for the *IL12A* rs568408 and *IL12A* rs2243115 polymorphisms. Thus, the reliability of the negative result of the present study was greater for IFN-γ than for IL-12 cytokine.

There are three possible explanations for the negative findings of the present study. First, the five polymorphisms included in this study may not alter the function of the *IFNγ*, *IL12A* or *IL12B* genes enough to influence the cytokine production, although the functionality of the two *IFNγ* and one of the three *IL12 SNP* has been well documented [5]. Second, homozygous variant genotypes may be needed to cause altered cytokine production, and the carriage of one mutated allele alone is not enough. In the present cohort, all except three of the variant *IL12A* and *IL12B* polymorphisms were heterozygous [5], whereas homozygous variations were common (18%) in the two included *IFNγ* polymorphisms [5]. Third, serum samples were obtained in adulthood years later than BCG osteitis manifested, and therefore, BCG vaccination complication could not be the stimulus that may be needed to trigger cytokine responses. The result concerning IFN-γ or IL-12 production in BCG-stimulated cell cultures was also negative [5], which may not be explainable by this time factor.

The negative results of the present study on the association of serum cytokine concentrations with the *IFNγ* rs2430561, *IFNγ* rs35314021, *IL12A* rs568408, *IL12A* rs2243115 and *IL12B* rs3212227 strengthen our previous conclusion that these five polymorphisms may not alter the respective genes enough to influence cytokine production [5]. Our 132 BCG osteitis patients with blood samples available is the largest BCG osteitis group ever published, but still, in general, small for genetic studies. Our originally 222 BCG osteitis patients form two-thirds of all 331 thus far globally published BCG osteitis cases [9].

Conclusion

The *IFNγ* rs2430561, *IFNγ* rs35314021, *IL12A* rs568408, *IL12A* rs2243115 and *IL12B* rs3212227 polymorphisms had no impact on IFN-γ and IL-12 serum concentrations. Previously in this same cohort of former BCG osteitis patients, these five polymorphisms had no impact on IFN-γ and IL-12 production in BCG-stimulated cell cultures. Since IFN-γ and IL-12 are no doubt involved in responses to BCG strains, other *IFNγ* and *IL12* polymorphisms need to be included in future studies on BCG vaccination and vaccination complications.

Bibliography

1. Kröger L., *et al*. "Osteitis after newborn vaccination with three different Bacillus Calmette-Guerin vaccines: twenty-nine years of experience". *Pediatric Infectious Disease Journal* 13.2 (1994): 113-116.

2. Kröger L., *et al*. "Osteitis caused by bacille Calmette-Guerin vaccination: a retrospective analysis of 222 cases". *Journal of Infectious Diseases* 172.8 (1995): 574-576.

3. Pöyhönen L., *et al*. "Interferon-gamma-dependent immunity in Bacillus Calmette-Guérin vaccine osteitis survivors". *Pediatric Infectious Disease Journal* 35.6 (2016): 690-694.

4. Abebe F. "Is interferon-gamma the right marker for bacille Calmette-Guérin-induced immune protection? The missing link in our understanding of tuberculosis immunology". *Clinical Experimental Immunology* 169.3 (2012): 213-219.

5. Korppi M., *et al*. "Interferon-γ and interleukin-12 production in relation to gene polymorphisms in BCG osteitis". *Pediatrics International* 61.10 (2019): 982-987.

6. Auton A., *et al*. "The 1000 Genomes Project Consortium. A global reference for human genetic variation". *Nature* 526.7571 (2015): 68-74.

7. Korppi M., *et al*. "Toll-like receptor 1, 2 and 6 polymorphisms: no association with 11 serum cytokine concentrations". *Acta Paediatrica* 107.12 (2018): 2217-2218.

8. Korppi M., *et al*. "TOLLIP rs5743854 and rs1169387 gene polymorphisms in Bacillus Calmette-Guerin osteitis". *EC Microbiology* 15.5 (2019): 377-381.

9. Lin WL., *et al*. "Management of Bacillus Calmette-Guerin osteomyelitis/osteitis in immunocompetent children - A systematic review". *Vaccine* 33.8 (2015): 4391-4397.

Volume 16 Issue 3 March 2020