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Research Article

Serum Interleukin (IL) -17A and IL-17F Concentrations in Relation to *IL17RA* Gene Variations in Bacillus Calmette-Guérin Osteitis Survivors

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Abstract

Aim: Interleukin-17 (IL-17) family cytokines are involved with the response to Bacillus Calmette-Guérin (BCG) vaccination, and IL-17A and IL-17F share a common IL-17 receptor A (IL-17RA). We evaluated the association of the *IL17RA* gene polymorphisms with serum IL-17A and IL-17F concentrations in adult BCG osteitis survivors.

Methods: Data on *IL17RA* rs4819553, rs4819554 and rs4819558 polymorphisms were available from 131 BCG survivors. The three included variations were in full linkage within a cluster of eight single nucleotide polymorphisms (SNPs). Variant *IL17RA* genotypes were present in 71.3%. In addition, Data on serum IL-17A and IL-17F concentrations were available from 129 adult BCG survivors. The present study is a secondary analysis combining these two datasets.

Results: There were no significant differences in serum IL-17A and IL-17F concentrations in either continuous or categorized analyses between cases with wild versus variant *IL17RA* genotypes. The categorized analyses were done using the detection limits, the outlier limits, and the limits of 25th, 50th and 75th percentiles. The small number of homozygous variant genotypes (3.1%) did not allow to study them separately.

Conclusion: *IL17RA* variations showed no association with IL-17A or IL-17F production in Finnish BCG osteitis survivors.

Keywords: *Bacillus Calmette Guérin; BCG Osteitis; Gene Polymorphism; Interleukin-17A; Interleukin-17F; Interleukin-17 receptor A*

Abbreviations

BCG: Bacillus Calmette Guérin; DNA: Deoxyribonucleic Acid; HRMA: High Resolution Melting Analysis; HPLC: High Performing Liquid Chromatography; IL-17A: Interleukin-17A; IL-17F: Interleukin-17F; IL-17RA: Interleukin-17 Receptor A (Protein); *IL17RA*: Interleukin17 Receptor A (Gene); mRNA: Messenger Ribonucleic Acid; PCR: Polymerase Chain Reaction; Th17: T Helper 17

Introduction

Interleukin-17 (IL-17) cytokines promote the host`s defense against many pathogens including mycobacteria [1] and affects to the responses to Bacillus Calmette Gu**é**rin (BCG) vaccination [2]. IL-17A and IL-17F are the most studied cytokines of the IL-17 family [3]. The

production of cytokines can be studied by determining their expression in cells or by measuring their concentrations in cell cultures or body fluids such as serum. Interleukin-17 receptor A (IL-17RA), encoded by the *IL17RA* gene, is a common receptor for IL-17A and IL-17F [4]. The IL-17RA seems to regulate the balance between promoting and inhibiting inflammation by IL-17A and/or by IL-17F [3].

We have previously found that the variations of the *IL17A* [5,6] or of the *1L17F* [7] genes were not associated with serum IL-17A or serum IL-17F concentrations, respectively, in adults with BCG osteitis in early childhood. We published recently the *IL17RA* genotype data of 132 BCG survivors, and based on the cluster of eight single nucleotide polymorphisms (SNPs), which were in full linkage, 68.7% of the cases had the wild, 28.2% the variant heterozygous and 3.1% the variant homozygous genotype [8]. The variant genotype may lead to reduced function of the receptor in question, and such reduction usually is more pronounced in carriers of variant homozygous than in carriers of variant heterozygous genotypes. Further, the reduced receptor function may increase cytokine concentrations in cell cultures or serum samples, as documented for example in interferon-gamma (IFN-γ)/IL-12 axis disorders [9].

Aim of the Study

The aim of this study was to evaluate the association of the *IL17RA* rs4819553, rs4819554 and rs4819558 SNPs, which belong to the cluster of eight wholly co-segregating SNPs, with the production of IL-17A and IL-17F cytokines. This study is a secondary analysis of two previously published datasets [5-8]. We compared serum IL-17A and IL-17F concentrations, which were measured in adults who presented with BCG osteitis after BCG vaccination in infancy, in relation to *IL17RA* wild versus variant genotypes.

Material and Methods

Subjects and samples

From 1960 to 1988, altogether 222 BCG osteitis cases were registered after newborn BCG vaccination in Finland, and the diagnosis was based on culture of the BCG strain and/or typical histology [10,11]. In 2007-2008, 132 former BCG osteitis patients aged now 21 - 47 years gave blood samples for further studies [12,13].

Whole blood samples were sent to the laboratory of the National Institute for Health and Welfare, Turku, Finland [12], where deoxyribonucleic acid (DNA) was isolated, and both DNA and serum samples were frozen at -70°C [13]. The frozen DNA and sera were transferred to the laboratory of Medical Microbiology and Immunology at the University of Turku, Turku, Finland, where the *IL17RA* rs4819553, rs4819554 and rs4819558 SNPs were determined in 131 samples [8] and serum IL-17A and IL-17F concentrations were measured in 129 samples [5-7].

IL17RA genotyping

High resolution melting analysis (HRMA) (Roche Diagnostics Light Cycler 480, Basel, Switzerland) was used for genotyping of the *IL17RA* rs4819554, as published recently [8].

In the 1000 Genomes Project linkage disequilibrium (LD) dataset, 18 SNPs in the promoter region of the *IL17RA*, including the *IL17RA* rs4819554, showed 100% co-segregation in most populations. Eight of those 18, including the *IL17RA* rs4819553 and rs4819958, had an identical allele frequency in the Finnish population. Therefore, we sequenced 25 samples of BCG osteitis cases to confirm the linkage between the three studied SNPs of *IL17RA* rs4819553, rs4819554 and rs4819558, and they were in full linkage [8].

In our cohort of 131 BCG osteitis survivors, the number of carriers of the wild *IL17RA* genotype was 90(68.7%), that of the variant het-

erozygous *IL17RA* genotype 37(28.2%) and that of the variant homozygous *IL17RA* genotype 4(3.1%) based on the data of the cluster of eight SNPs in the gene [8].

Functionality of the *IL17RA* rs4819553, rs4819554 and rs4819558

A previous study [14] demonstrated that IL-17RA protein and specific messenger ribonucleic acid (mRNA) expressions of blood mononuclear cells including T helper 17 (Th17) cells were increased in subjects who were homozygous for the major alleles of the *IL17RA* rs4819553 and rs4819554, compared with those who carried the minor alleles. The *IL17RA* rs4819958 was not included in the study, but due to full linkage, the functionality data can be expected to see similar.

Serum IL-17A and IL-17F concentrations

Serum IL-17A concentrations in this cohort and the methods for measurements were published previously [5,6]. In short: Bio-Plex Pro human IL-10 immunoassay kit was used to measure serum concentrations using the Bio-Plex 200 System (Bio-Rad, Helsinki, Finland). The detection limit of the method for IL-17A concentration was 2.5 pg/mL.

Serum IL-17F concentrations were measured, as recently published [7], using a commercial ELISA kit (DuoSet ELISA, Human IL-17F, R&D systems, Abingdon, the UK). Optical density was measured, and standard curve analysis was done with GraphPad prism 4.0 version (San Diego, CA, the USA). The detection limit of the method for IL-17F concentration was 12.5 pg/mL.

Statistics

The Statistical Package of SPSS for Windows, version 23 (IBM Corp, Armonk, NY, USA) was used for statistical analyses. Exploratory data analyses revealed that IL-17A and IL-17F concentrations were non-normally distributed, and therefore the Mann-Whitney U test was used to compare the concentrations between study subjects with wild and variant *IL17RA* genotypes. The results are given as medians, interquartile (IQ) 25% - 75% ranges and minimum to maximum ranges. Chi square and Fisher´s exact tests, as appropriate, were used in categorized analyses. The limits for the outliers were: Q3 (25%) + 1.5 x IQ (outlier) and Q3 (75%) + 3 x IQ (extreme outlier).

Ethics

The study was approved by the Ethics Committee of the Tampere University Hospital District, Tampere, Finland. Former BCG osteitis patients gave their voluntary, written informed consent for further studies including permission to perform immunological and genetic studies concerning susceptibility to BCG vaccination complications. The laboratory work was done without personal information using coded samples.

Results

As seen in figure 1, median serum concentration of IL-17A was 51.7 pg/mL (IQ 13.5 - 53.8) in the 89 subjects with the wild genotypes of the *IL17RA* gene and 34.8 pg/mL (14.2 - 80.3) in those 40 with the variant hetero- or homozygous genotype ($p = 0.215$). The IL-17A concentration was < 2.5 pg/mL (detection limit) in 6.7% of those with the wild and in 1.5% of those with the variant genotype ($p = 0.435$).

As seen in figure 2, the median serum concentration of IL-17F was 25.3 pg/mL (IQ < 1.22 - 194.5) in the 89 subjects with the wild genotypes of the *IL17RA* gene and 14.2 pg/ml (< 1.22 - 196.7) in those 40 with the variant hetero- or homozygous genotype ($p = 0.337$). The IL-17F concentration was < 12.5 pg/mL (detection limit) in 38.2% of those with the wild and in 52.5% of those with the variant genotype ($p = 0.178$).

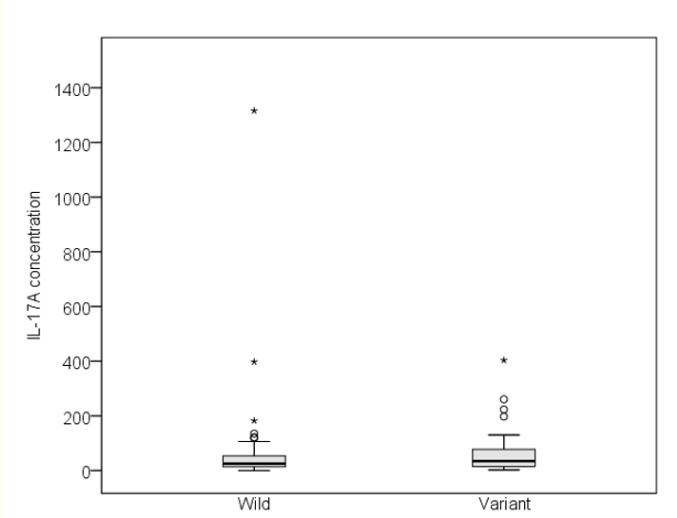

Figure 1: *Serum IL-17A concentrations (pg/ml) in 129 former BCG osteitis patients in relation to the wild and variant genotypes of the IL17RA rs4819553, rs4819554 and rs4819558 polymorphisms.*

*The box-plot figure expresses medians and quartiles (Q1, Q3). There were two outliers (Q3 + 1.5 x interquartile range) expressed as small circles O and five extreme outliers (Q3 + 3 x interquartile range) expressed as stars *. One extreme outlier (wild) is situated outside the figure. Statistical significance: p = 0.215.*

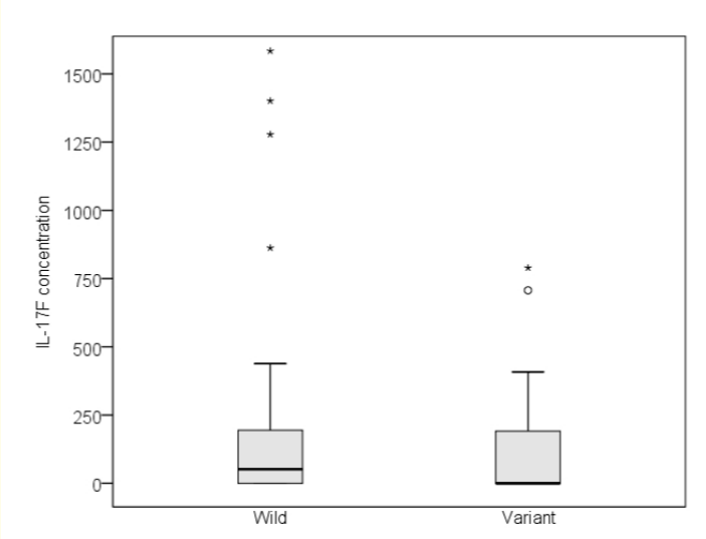

Figure 2: *Serum IL-17F concentrations (pg/ml) in 129 former BCG osteitis patients in relation to the wild and variant genotypes of the IL17RA rs4819553, rs4819554 and rs4819558 polymorphism.*

*The box-plot figure expresses medians and quartiles (Q1, Q3). There were two outliers (Q3 + 1.5 x interquartile range) expressed as small circles O and five extreme outliers (Q3 + 3 x interquartile range) expressed as stars *. Two extreme outliers are situated outside the figure. Statistical significance: p = 0.337.*

When the IL-17A and IL-17F data were categorized at the cut-off limits of 25th, 50th or 75th percentiles, the result remained negative (Data not shown). The outliers which represent exceptionally high values, were present for IL-17A in 6.7% (wild) versus 10.0% (variant) (p = 0.522) and for IL-17F in 5.6% (wild) versus 7,5% (variant) (p = 0.703). Thus, the *IL17RA* gene variations were not associated either with low nor with high serum IL-17A and IL-17F concentrations.

Discussion

The main result of this study was that serum IL-17A and IL-17F concentrations, when measured in adult BCG osteitis survivors, did not differ between those with wild and those with variant genotypes of the three *IL17RA* polymorphisms. The result is in line with our previous observations that *IL17A* and *IL17F* gene variations showed no association with serum IL-17A [5,6] or IL-17F [7] concentrations in this same cohort. Our expectation was that *IL17A* and *IL17F* variations may decrease the production of respective cytokines, and correspondingly, *IL17RA* variations may, because of weakened IL-17RA receptor function, lead to higher serum IL-17A and IL-17F concentrations. This kind of connection between receptor function and cell culture or serum cytokine concentrations has been observed for example in monogenic Mendelian susceptibility to mycobacterial diseases (MSMD) [9].

There are two possible explanations for our negative result on the association of the *IL17RA* rs4819553, rs4819554 and rs4819558 variations with serum IL-17A and IL-17F concentrations. An increase in the cytokine concentration may need a stimulus, such as an ongoing infection or an exacerbation of an inflammatory disease, which were not allowed in our cohort during blood sampling [12,13]. When IFN-γ or IL-12 productions are used in the diagnosis of MSMD disorders, the cells or whole blood samples need to be stimulated by BCG [15]. Naturally, BCG osteitis in early childhood cannot anymore be such a stimulus years later in adulthood.

The second possibility is that the selected polymorphisms do not alter the function of the *IL17RA* gene enough to influence the IL-17A or IL-17F concentrations in serum. In the IFN- γ/IL-12 loop, for example, reduced receptor functions lead to high serum IFN-g or IL-12 concentrations, respectively [9]. Homozygous mutations are usually associated with complete and heterozygous mutations with partial reductions in the receptor function. Likewise, the emergence of IL-17RA deficiency leading to chronic mucocutaneous candidiasis needed that patients were homozygous for at least one of the variant alleles in the *IL17RA* gene [16] and the *IL17RA* rs4819553 and rs4819554 were associated with *ex vivo* IL-17A protein and mRNA production only if the subjects were homozygous [14]. In the present study, only 3.1% of the cases were homozygous for the studied *IL17RA* variations, which means that they could not be analyzed separately in relation to IL-17A or IL-17F production.

In this study, the concentrations of serum IL-17A and IL-17F cannot be directly compared, because the used methods were different with different detection limits.

Conclusion

In conclusion, *IL17RA* promoter-region gene polymorphisms were not associated with the non-stimulated serum concentrations of IL-17A and IL-17F in Finnish BCG osteitis survivors.

Conflicts of Interest

The authors reported no conflicts of interest to declare.

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