Human Immunology 75 (2014) 91–97

Contents lists available at ScienceDirect

journal homepage: www.elsevier.com/locate/humimm

Associations between cytokine receptor polymorphisms and variability in laboratory immune parameters in normal humans

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ARTICLE INFO

Article history:
Received 7 January 2013
Accepted 11 September 2013
Available online 20 September 2013

ABSTRACT

In every study involving human immune parameters, large inter-subject variability occurs which can make interpretation of results difficult. The aim of this study was to evaluate whether genetic variants in cytokine receptors could associate with variability in laboratory immune measures. A total of 207 normal volunteers were recruited in this study. Immunoregulatory profiles were measured by flow cytometry and genotyping assays were performed by allelic discrimination real-time PCR. Immunoregulatory profiles were categorized according to various single nucleotide polymorphisms (SNPs) of cytokine receptors including T-56C and G-611A of IFN-γ receptor 1 (IFNGR1); Q64R of IFNGR2; and Ile50Val, Q576R and S503P of IL4R. Results reveal that Th1 levels were significantly higher in the heterozygous of the IFNGR1 T-56C polymorphism (minor allele) compared to wild-type (WT, major allele) ($p = 0.006$). For the Q576R of IL4R, Th1/Th2 ratio was significantly lower for the homozygous SNP (Arg/Arg) compared to the WT (Gln/Gln) ($p = 0.035$). In addition, the significant interaction effects of demographic characteristics on SNP-immune parameter associations were reported as well. We conclude that cytokine receptor polymorphisms might associate with variability in laboratory immune measures. Approach of SNP analysis of cytokine receptors can be useful in categorizing baseline immune responses to more accurately evaluate clinical immune data.

1. Introduction

Cytokines are pleiotropic in their biological activities and play pivotal roles in a variety of responses, including the immune response, hematopoiesis, neurogenesis, embryogenesis, and oncogenesis via binding specific cell-surface receptors on their target cells. Immune deviation is primarily concerned with the balance between cytokines produced by T helper 1 (Th1) and Th2 cells [1–3]. Th1 cells produce interferon-γ (IFN-γ), IL-2, and TNF-β which promote cell-mediated immunity against intracellular pathogens, whereas Th2 cells produce IL-4, IL-5, IL-9, and IL-13 which promote humoral immunity against extracellular pathogens [4–7]. Select cytokines regulate the subpopulation of T-cell lymphocytes responsible for this balance. IFN-γ is produced by effector T and natural killer cells, and is vital for host defense against viral infection. Both IL-4 and IL-13 are important Th2 cytokines that trigger isotype switching of B cells to produce IgE [8,9].

Gene expression of cytokines and cytokine receptors is tightly regulated, and aberrant expression from environmental and genetic factors has been implicated in a range of diseases, susceptibility to infections, and responses to treatment [10]. Although most SNPs are not functionally important, there is a subset of variants that alter the expression or function of a gene product [11]. These functional variants may alter disease risk, affect the observed phenotype, contribute to the pathogenesis of the disease, or alter the response to treatment [12–14]. A previous study demonstrated that the variant of IFNGR1 T-56C (rs2234711) was associated with high susceptibility to *Helicobacter pylori* infection, protection against severe malaria, and pulmonary tuberculosis [15–17]. Rosenzweig et al. demonstrated SNP at position -611 (rs1327474) of IFNGR1 had a stronger effect on the promoter activity than those at position -56 of IFNGR1 [18]. It was also reported that Q64R (rs2070385) of IFNGR2 is a fully functional polymorphism in an *in vitro* study [19] and associated with total IgE levels [20]. Furthermore, several IL4R polymorphisms, such as Ile50Val (rs1805010), Q576R (rs1801275) and S503P (1805015), have been demonstrated to be associated with a higher risk of atopy and/or atopic asthma [21–23].

The large variability in baseline immunological parameters between individuals makes diagnosis and management of immune-based diseases challenging [24]. For example, in one study

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0198-8859/$36.00 - see front matter © 2013 American Society for Histocompatibility and Immunogenetics. Published by Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.humimm.2013.09.007

measuring the number of regulatory T cells (Treg) in atopic and non-atopic subjects, the percent of Treg in the peripheral blood ranged from 2.1% to 19.8%, nearly a 10-fold difference [25]. Cytokines receptor polymorphisms have been found to significantly influence various cell responses to cytokines. Additionally, the expression of Th1 and Th2 cytokines can be altered by germ-line genetic variants [26]. Thus we investigated the associations between gene polymorphisms in IFN-γR1, IFN-γR2 and IL4R and immune measures. In addition, effects of demographic characteristics, including age, gender, race, and BMI, on cytokine receptor polymorphisms-immune measures associations were analyzed. The aim of the study was to evaluate whether genetic variants in cytokine receptors could associate with variability in laboratory immune parameters and search for stable biomarkers that could have potential utility in diagnosis and/or management of immune-associated diseases.

2. Materials and methods

2.1. Human subjects

A total of 207 healthy volunteers were recruited after obtaining written informed consent according to a University of Mississippi Medical Center IRB-approved protocol. Subjects were excluded for any past medical history of smoking, psychiatric illnesses, cardiovascular diseases, autoimmune diseases, or recent medical use of glucocorticoids, catecholamines, and/or adrenergic antagonists .Allergic status was categorized by self-report of symptoms consistent with allergic rhinitis [27].

2.2. Blood collection and PBMC isolation

Venous blood was collected into heparinized tubes for peripheral blood mononuclear cells (PBMC) isolation, and into EDTA coated tubes for genetic assessment. PBMC were isolated using a Ficoll–Hypaque gradient as previously described [28]. Briefly, cells were centrifuged at 650×*g* for 30 min, washed twice in HBSS, and then resuspended in 1 ml cRPMI supplemented with 5% FBS. Cell counts were obtained using an automated hematology analyzer (Coulter).

2.3. Flow cytometry

T cell populations in PBMC were analyzed by flow cytometry according to previously described methods [29]. Briefly, 1×10^6 PBMC were incubated with the following surface antibodies: CD3-APC, CD4- or CD8-PerCP, or CD25-PE. Cells were then washed, fixed and incubated with the following intracellular antibodies: FoxP3-FITC, IL10-FITC, or TGFβ-PE. For the detection of IFNγ and IL4, cells were first stimulated with 10 ng/ml phorbolmyristate acetate (PMA), 1 μg/ml ionomycin, and 3 μM monensinfor 4 h at 37 °C and 5% CO_2. Stimulated cells were then fixed with 4% PFA for 10 min at RT, washed, permeabilized as described above, and incubated with CD3-APC, CD8-Per-CP, IL4-PE, and IFNγ-FITC for 1 h at RT. Appropriate isotype controls were used to define positive and negative staining during the initial set-up of the T cell profiles of interest. Data were collected using the BD FACSCalibur flow cytometer and analyzed with the CellQuest software. Cell populations were defined as follows, Th1: $CD3^+CD8^-IFN\gamma^+IL4^-$; Th2: $CD3^+CD8^-IFN\gamma^-IL4^+$; Treg: $CD3^+CD4^+CD25^{hi}FoxP3^+$; Tr1: $CD3^+CD8^-IL10^+$; Th3: $CD3^+CD8^-TGF\beta^+$.

2.4. Genomic DNA preparation and genotyping assay

DNA was extracted from whole blood sample using commercial Puregene DNA Isolation Kit (Centra Systems) and 20 ng of DNA was used in each assay. Genotyping assays for T-56C and G-611A of IFNGR1, Q64R of IFNGR2, and Ile50Val, Q576R and S503P of IL4R were performed by allelic discrimination real-time PCR using Applied Biosystems 7300 Real-Time PCR System with TaqMan SNP assay reagents and genotyping analyzed by SDS 2.1 package (Applied Biosystems, Foster City, CA, USA). Pre-designed primer/probe sets were available from Applied Biosystems (Table 1).

2.5. Statistical analysis

Hardy–Weinberg tests for equilibrium were analyzes using the χ^2 test. Descriptive statistics were computed as means (95% confidence intervals (CI)) for normally distributed data and geometric means (95% CI) for skewed data for continuous variables and frequency counts and percentages for categorical variables. Analyses were performed after log transformations were applied to skewed data (Th1/Th2 ratio, Treg, Tr1, and Th3) based on the exploratory data analyses. Main effects of SNPs, as well as their interactions with demographic variables on immune parameters were examined using the linear regression analyses. Models were adjusted for gender, race, allergic status, age, BMI, and their interactions. We used likelihood ratio tests to assess interaction effects in nested models. Geometric means with 95% CI and relative changes (exponentiated parameter estimates) from models were reported for log-transformed outcomes.

Due to the limited number of participants in the homozygous SNP group for Q64R polymorhism (Arg/Arg) of IFNGR2 (3% of the sample), comparison was made between Gln/Gln and Gln/Arg groups. Similarly, for the S503P polymorphism of IL4R, comparison was made between the heterozygous (Ser/Pro) SNP and WT (Ser/Ser) due to small number of participants in the Pro/Pro subgroup (5.4%). Sensitivity analyses examining potentially influential data points were conducted. A subject with high Th2 (11.21%) and a subject with high Th1 (67.29%) were excluded from the analyses. Additionally, two individuals with the highest Th1/Th2 ratio scores (97.48 and 78.5) and one individual with the lowest Tr1 (0.04) were excluded for Th1/Th2 ratio and Tr1 analyses, respectively as influential points. Results without these outliers were reported. Approximate normal residuals were obtained via diagnostic model assessments. Stata (version 12.1; StataCorp, College Station, TX, USA) was used to perform statistical analyses. The significance level for two-sided hypothesis testing was set at 0.05.

3. Results

3.1. Sample description

Average age of 207 healthy volunteers was 35.28 ± 12.22 with a range of 16–64. The participants included 55 males (26.6%) and

Table 1
Probe sequences of cytokine receptor polymorphisms.

Gene	SNP	dbSNP ID	Probes for Taqman Analysis
IFNGR1	T-56C	rs2234711	5′-FAM-AGCCCAGC**G**CTGCCCTCCA
			5′-VIC-CAGCCCAGC**A**CTGCCCTCCA
	G-611A	rs1327474	5′-FAM-ATCAGTTTAT**T**AGGCAGCCT
			5′-VIC-TCAGTTTAT**C**AGGCAGCCTC
IFNGR2	Q64R	rs2070385	5′-FAM-AACTGCACT**C**GGTAGA
			5′-VIC-TAAACTGCACT**T**GGTAGAC
IL4R	Ile50Val	rs1805010	5′-FAM-CACACGTGT**A**TCCCTGAGA
			5′-VIC-CACACGTG T**G**TCCCTGAGA
	Q576R	rs1801275	5′-FAM-GTGGCTATC**A**GGAGTTTGT
			5′-VIC-GTGGCTATC**G**GGAGTTTGT
	S503P	rs1805015	5′-FAM-TTCAGCAAC**T**CCCTGAGCC
			5′-VIC-CTTCAGCAAC**C**CCCTGAGCC