Human Immunology 73 (2012) 636–640

Contents lists available at SciVerse ScienceDirect

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

Rapid Communication

Replication of associations between cytokine and cytokine receptor single nucleotide polymorphisms and measles-specific adaptive immunophenotypic extremes

Sarah J. White [a,c], Iana H. Haralambieva [a,c], Inna G. Ovsyannikova [a,c], Robert A. Vierkant [b], Megan M. O'Byrne [b], Gregory A. Poland [a,c,*]

[a] Mayo Clinic Vaccine Research Group, Mayo Clinic, 200 First Street SW, Rochester, MN 55905, United States
[b] Division of Biostatistics, Mayo Clinic, 200 First Street SW, Rochester, MN 55905, United States
[c] Program in Translational Immunovirology and Biodefense, Mayo Clinic, 200 First Street SW, Rochester, MN 55905, United States

ARTICLE INFO

Article history:
Received 5 December 2011
Accepted 19 March 2012
Available online 12 April 2012

ABSTRACT

Our objective was to replicate previously reported associations between cytokine and cytokine receptor SNPs and humoral and CMI (cell-mediated immune) responses to measles vaccine. All subjects ($n = 758$) received two doses of MMR (measles/mumps/rubella) vaccine. From these subjects, candidate cytokine and cytokine receptor SNPs were genotyped and analyzed in 29–30 subjects falling into one of four "extreme" humoral ($Ab^{high/low}$) and CMI ($CMI^{high/low}$) response quadrants. Associations between seven SNPs (out of 11 in the discovery study) and measles-specific neutralizing antibody levels and IFN-γ ELISPOT responses were evaluated using chi-square tests. We found one replicated association for SNP rs372889 in the *IL12RB1* gene ($P = 0.03$ for $Ab^{high}CMI^{high}$ vs. $Ab^{low}CMI^{low}$). Our findings demonstrate the importance of replicating genotypic-phenotypic associations, which can be achieved using immunophenotypic extremes and smaller sample sizes. We speculate that *IL12RB1* polymorphisms may affect IL-12 and IL-23 binding and downstream effects, which are critical cytokines in the CMI response to measles vaccine.

1. Introduction

A resurgence of measles outbreaks, along with other vaccine-preventable diseases (i.e., mumps and rubella), have been reported worldwide. In fact, from 2001 to 2008 a median of 56 confirmed measles cases was reported annually in the United States, and between January and September 15, 2011, 211 confirmed cases of measles were reported [1]. Eighteen of the 211 cases were in people who had received one or more doses of the MMR (measles/mumps/rubella) vaccine. Hence, primary vaccine failure and waning immunity limit protection against the disease in certain vaccinated individuals. This is attributed in large part to inter-individual genetic variation of immune responses [2–4].

Innate and adaptive measles immune responses are genetically influenced as evidenced by the heritability of measles antibody response of 89% [2]. Specific polymorphisms of cytokines and their receptors have been shown to alter cytokine levels and/or activity, impacting cytokine function and potentially the overall balance of Type I helper T cell (Th1)/Th2/Th17 cytokines [5]. Cytokines and their receptors are essential to measles-induced immunity following either contact with the wild-type viral strain (infection) or the live Edmonston–Enders viral vaccine strain [6,7]. Therefore, cytokine and cytokine receptor gene polymorphisms, especially in coding and promoter regions, may increase (or decrease) one's susceptibility to measles infection and/or their response to measles vaccine.

Identification of associations between cytokine and cytokine receptor gene polymorphisms and variations in humoral and CMI responses provides information regarding the use of cytokines as markers of immune response [8] or as potential adjuvants [9] in new measles vaccines. Replication of these associations, using an independent population from the discovery study, is necessary to differentiate true genetic associations from Type I statistical errors [10].

While large sample sizes are typically required [11] for association studies, sufficient power can be attained with a substantially smaller sample size by selectively genotyping individuals with phenotypic immune "extremes" [12]. The objective of this study was to validate previously reported discovery associations

Abbreviations: CMI, cell-mediated immunity; MMR, measles/mumps/rubella; Ab, antibody; CDC, centers for disease control and prevention; Th1, helper T cells.

* Corresponding author at: Mayo Clinic Vaccine Research Group, Mayo Clinic, Guggenheim 611C, 200 First Street SW, Rochester, MN 55905, United States. Fax: +1 507 266 4716.
E-mail address: Poland.Gregory@mayo.edu (G.A. Poland).

0198-8859/$36.00 - see front matter © 2012 American Society for Histocompatibility and Immunogenetics. Published by Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.humimm.2012.03.015

between cytokine and cytokine receptor SNPs and humoral and CMI responses using a sub-cohort of children and young adults with extreme humoral and cell-mediated immune responses.

2. Materials and methods

2.1. Subjects

Briefly, study subjects from a preceding discovery [13] and a current replication study were selected from two independent cohorts of previously recruited, randomly selected healthy children and young adults from all socioeconomic backgrounds in Olmsted County, MN, who had been immunized with two doses of MMR vaccine. The discovery cohort included 346 eligible subjects between 12 and 18 years of age (93% Caucasians), and the current replication cohort included 758 separate, eligible subjects between 11 and 22 years of age (91% Caucasians). No known wild-type measles virus had been reported within the geographical area for each of the subject's lifetime. All human studies were approved by the institutional review board of the Mayo Clinic and informed consent was obtained. Further details regarding study enrollment can be found elsewhere for the original discovery [14] and replication study cohorts [15].

Measles vaccine-specific humoral and CMI responses were quantified by a measles-specific IgG enzyme immunoassay (Dade Behring, Marburg, Germany) [17] and a lymphoproliferation assay (SI $\geqslant$ 3 indicates positive response) [13] (respectively) for the 346 subjects in the discovery cohort; and a measles virus plaque reduction neutralization (PRN) assay [16,17] and an IFN-γ enzyme-linked immunosorbent spot (ELISPOT) assay [18] (respectively) for the 758 subjects in the replication cohort. These methods reflect differences in gold-standard methods, at the time, for measuring humoral and CMI responses in each cohort.

The PRN assay was performed using serial dilution replicates of heat-inactivated sera samples and standards (3rd WHO international anti-measles antibody standard). The GFP-positive plaques were scanned and counted using high-throughput fluorescent microscopy; and their titers were transformed to mIU/ml units using the 3rd WHO standard. A neutralizing antibody titer of $\geqslant$210 mIU/ml, corresponding to a PRN titer of 120 mIU/ml [16], was considered indicative of seroprotection. Low variability (CV = 5.7%) was found based the standard values, which also served as a part of the quality assurance/quality control efforts. Further details are described elsewhere [16,17].

For the IFN-γ ELISPOT assay, subjects' peripheral blood mononuclear cells (PBMC) were stimulated with measles vaccine virus (MOI = 0.05; 42 h incubation) and plated in triplicates onto commercially available kits (R&D Systems, Minneapolis, MN). Positive (stimulation with PHA) and negative (un-stimulated) controls were used for each subject. Further details are as previously described [18].

In our initial discovery study [13] and the current replication study, a sub-cohort of subjects with immunophenotypic extremes based on humoral and cell-mediated immune response to measles vaccine were identified. For each cohort, scatterplots of log-transformed humoral and cell-mediated immune response measures were generated. A two-step quadrant selection approach based on these scatterplots was performed to identify study subjects falling into one of four "extreme" adaptive immune response quadrants: $Ab^{low}CMI^{low}$, $Ab^{low}CMI^{high}$, $Ab^{high}CMI^{low}$ and $Ab^{high}CMI^{high}$. First, subjects with either humoral or cell-mediated immune response values falling within the middle 50% of all values were removed from the sampling pool. Second, of the remaining subjects, the squared distance from each of the transformed variables and the intersection of the horizontal and vertical median line within the corresponding quadrant was calculated. The 30 subjects with the largest squared distances in each quadrant were characterized as phenotypic "extremes" and were selected as the sub-cohorts for use in this study. It is worth mentioning that the final sub-cohort sample size selected for the preceding discovery study (1st cohort) was 118 subjects (n = 29–30 per quadrant) because two subjects did not provide informed consent to be tested in subsequent vaccine-related studies. The final sub-cohort sample size selected for the current replication study (2nd cohort) was 120 subjects (n = 30 per quadrant). Subject selection using this quadrant approach is depicted in Fig. 1.

2.2. SNP genotyping

Fifty-eight tag SNPs in candidate genes were originally selected for genotyping in the phenotypic extremes of the 1st cohort. Selection criteria consisted of a minor allele frequency of $\geqslant$0.05 located within a particular loci (coding region or 5′ or 3′ untranslated region or intron), and a previous genotype:phenotype association. Further details have been previously published [13]. Prior to genotyping, genomic DNA was first extracted from clotted blood with the Puregene extraction kit (Gentra Systems, Minneapolis, MN) and DNA concentrations were measured using the Picogreen method (Molecular Probes, Carlsbad, CA) for the discovery and the current studies. Custom Illumina GoldenGate SNP panels were used for the 768 total SNPs. Quality control measures included replicates of genomic DNA sequence standards (e.g., CEPH standards from Coriell), which were used to refine clustering, and replicates without DNA. In addition, subjects with low call rate SNPs (<0.95) were removed from analysis.

For the current study, seven SNPs were successfully genotyped and analyzed using custom Illumina GoldenGate SNP panels, as described previously [14,15]. While 11 cytokine and cytokine

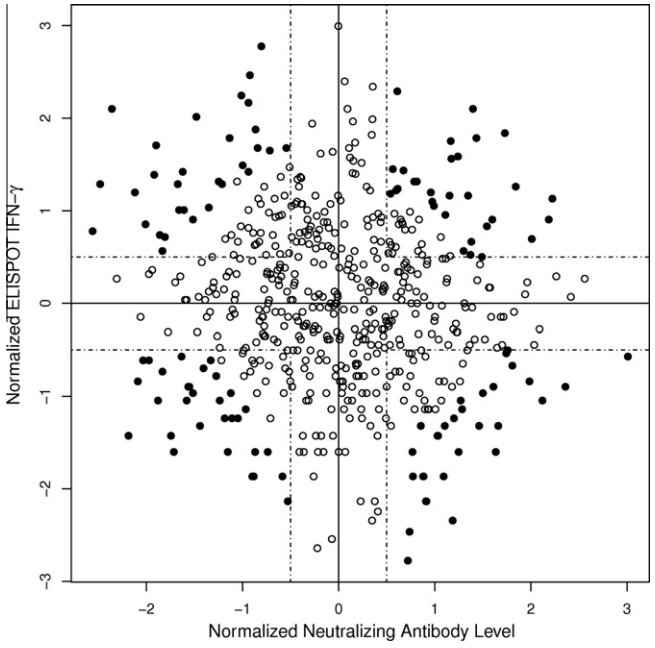

Fig. 1. Selection of a set of study subjects based on a measles vaccine-specific humoral (neutralizing antibody levels) and cell-mediated (quantitation of IFN-γ-secreting lymphocytes) immune response. A (●) represents the 30 subjects with the most extreme phenotypes in each quadrant, whereas a (○) represents the remaining subjects not included in the SNP genotyping or statistical analyses. Horizontal and vertical solid lines represent the median lines for the cell-mediated and humoral immune responses (respectively) in each quadrant. Horizontal and vertical dashed lines represent the upper and lower IQR for the cell-mediated and humoral immune responses (respectively) in each quadrant.