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Influence of cytokine gene variations on immunization to childhood vaccines☆

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ABSTRACT

The magnitude of the immune response to vaccinations can be influenced by genetic variability. In the present study, we aimed to investigate whether cytokine or cytokine receptor gene polymorphisms were associated with variations in the immune response to childhood vaccination. The study group consisted of 141 healthy infants who had been immunized with hepatitis B vaccine (HBV), 7-valent pneumococcal conjugate (PCV7), and diphtheria, tetanus, acellular pertussis (DTaP) vaccines according to standard childhood immunization schedules. Genotype analysis was performed on genomic DNA using a 5′ nuclease PCR assay. Post vaccination total, isotypic, and antigen-specific serum antibody levels were measured using multiplex immunoassays. Significant associations were observed between SNPs in the TNFα, IL-12B, IL-4Rα, and IL-10 genes and vaccine-specific immune responses ($p < 0.05$). In addition, SNPs in the IL-1β, TNFα, IL-2, IL-4, IL-10, IL-4Rα, and IL-12B genes were associated with variations in serum levels of immunoglobulins (IgG, IgA, IgM) and IgG isotypes (IgG1–IgG3) ($p < 0.05$). These data suggest that genetic variations in cytokine genes can influence vaccine-induced immune responses in infants, which in turn may influence vaccine efficacy.

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1. Introduction

Despite the use of uniform administration schemes, there is considerable inter-individual variation in immune responses to vaccines. For example, 5–20% of vaccinated individuals experience either hypo- or non-responsiveness to hepatitis B vaccine (HBV) and 2–10% fail to produce protective levels of measles antibodies [1,2]. Subsequently, these individuals are at increased risk of developing vaccine preventable infectious diseases and, if identified, would benefit by the implementation of an alternative vaccination schedule. Several mechanisms have been proposed to explain hypo- or non-responsiveness including defects in T- and B-cell functions, antigen processing and presentation, cytokine secretion profiles, and immunologic tolerance which are in turn influenced by a complex interplay between genetic, physiological and environmental factors [3–5].

Regarding the genetic influences on inter-individual differences in vaccine responses, most studies have focused on human leukocyte antigen (HLA) alleles and Ig allotypes [6–8]. The influence of genetic variations in other immune-related genes, such as those that influence expression of cytokines, cytokine receptors or toll-like receptors have also been studied to some extent [9–13]. For example, 60% of genetic variability in the immune response to hepatitis B surface antigen (HBsAg) has been attributed to non-HLA genes [14]. Cytokines and cytokine receptors play central roles in homeostatic, pathologic, and protective immune responses, including the regulation of the Th1/Th2 balance in response to vaccine antigens [4,15,16] The role of cytokines in controlling various aspects of the immune response has been used to optimize vaccine efficacy. For example, stronger immune responses have been noted when given in the presence of IL-2, IL-10, or IL-12 [17–19]. Genetic variations in cytokine genes can disrupt their transcriptional rates or affect their expression and function. Single nucleotide polymorphisms (SNPs) in cytokine genes such as IL-1, IL-4, IL-6, and IL-10 have been associated with susceptibility to and severity of infectious diseases and are known to influence vaccine-induced immune responses [20,21]. We have previously shown that the IL-1β +3953

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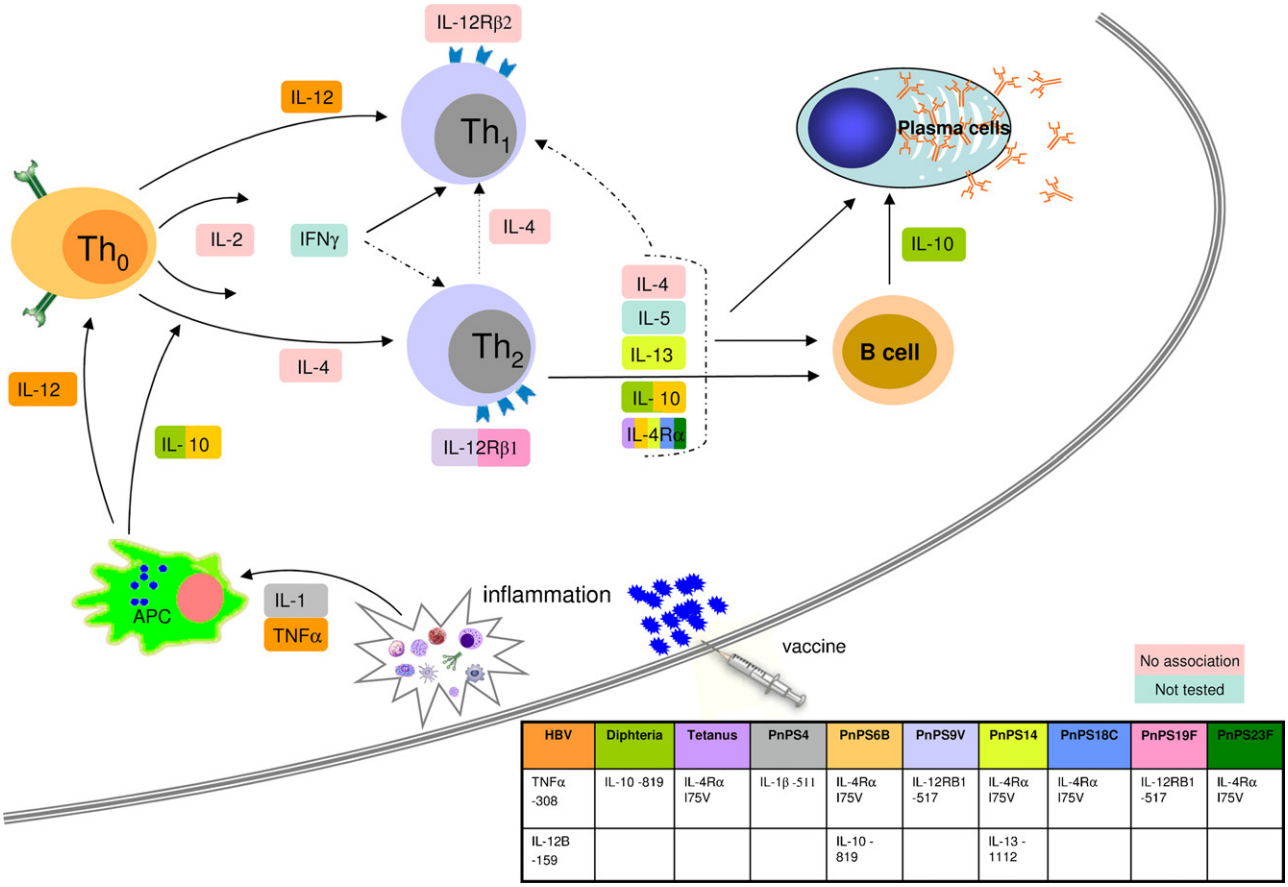

HBV	Diphteria	Tetanus	PnPS4	PnPS6B	PnPS9V	PnPS14	PnPS18C	PnPS19F	PnPS23F
TNFα -308	IL-10 -819	IL-4Rα I75V	IL-1β -511	IL-4Rα I75V	IL-12RB1 -517	IL-4Rα I75V	IL-4Rα I75V	IL-12RB1 -517	IL-4Rα I75V
IL-12B -159				IL-10 -819		IL-13 -1112			

Fig. 1. Simplified network of cytokines in the immune process of vaccine antigens and the main results of this study. Solid arrows indicate positive and dashed arrows indicate negative influences.

variant is associated with variations in antibody and T-cell lymphoproliferative responses to HBV in adults [22]. Genetic variations in the IL-2, IL-4, and IL-12B genes were also reported to be associated with the immune response to HBsAg, independent of HLA and other cytokine gene variants [23]. IL-4 and IL-4Rα SNPs were found to influence anti-pneumococcal response and IL-10 SNPs were associated with altered response to HBsAg and inactivated influenza vaccine [10,24,25]. SNPs in the IL-2, IL-10, and IL-12RB genes were also associated with variations in measles vaccine-induced immunity [26]. These findings have potential implications for developing vaccine candidates that incorporate cytokines (e.g., cytokine adjuvants) to maintain an optimal Th1/Th2 balance in recipients with diverse genetic background.

The present study was designed to investigate whether genetic variations in cytokine and cytokine receptor genes contribute to variations in vaccine-induced immune responses in early life following immunization with HBV, 7-valent pneumococcal conjugate vaccine (PCV7), and diphtheria, tetanus, and pertussis vaccine (DTaP). In addition to the pro-inflammatory cytokines IL-1 and TNFα, genetic polymorphisms in the genes encoding cytokines representing both subsets of Th1 (IL-2, IL-2RA, IL-12p40, IL-12RB1, IL-12RB2) and Th2 (IL-4, IL-4Rα, IL-10, IL-13) were studied. All the SNPs tested are known or suspected to alter the function of the respective gene. As depicted in Fig. 1, the selected genes and their interactions are crucial for the efficient generation of antigen-specific immune responses. They are involved in inflammation, antigen presenting cell functions, Th1/Th2 polarization, proliferation and differentiation of T- and B-cells, and regulation of antibody responses. Therefore, the analyses were conducted on the basis of general assumptions derived from currently accepted roles of cytokines in immune processes. Major serum immunoglobulin classes (IgG, IgA, and IgM) and IgG subclasses (IgG1–G4) were also measured and genotype specific variations in their levels were assessed. Igs are key components of the humoral immune system and their levels reflect not only immune development but humoral immune function as well.

2. Materials and methods

2.1. Study population and vaccinations

Study procedures were approved by the Institutional Review Boards of all participating institutions. The subjects were infants seen in two University-affiliated general pediatrics clinics for routine 1-year-old check up examinations. These clinics routinely obtained blood by finger stick during the 1-year check up to screen for anemia and lead poisoning. If parents gave informed consent, additional tubes of coagulated blood for serum and EDTA treated whole blood for genomic DNA were obtained. In addition, immunization records were reviewed to document appropriate history of immunization with HBV (Recombivax®, Merck&Co., Inc., Whitehouse Station, NJ); DTaP (Daptacel®, Sanofi Pasteur, Ontario, CA); heptavalent pneumococcal conjugate vaccine (PV7) (Prevnar®, Wyeth, Philadelphia, PA); inactivated polio vaccine (IPV); and *Haemophilus influenzae* type b (Hib) conjugate vaccine in accordance with then-current guidelines for childhood immunization [27]. A total of 141 healthy infants, aged 11.5–14 months (mean: 12.5 months), were recruited into the study. The majority of children (84%) were non-Hispanic whites, 58% were male.

Table 1
Genes and SNPs analyzed for association with vaccine-induced immune response.

Gene	Position	SNP ID[a]
TNFα	−308	rs1800629
	−238	rs361525
IL-1β	−511	rs16944
	+3953	rs1143634
IL-2	−34	rs2069763
	−384	rs2069762
IL-2RA	−17245	rs706781
IL-4	−590	rs2243250
	−33	rs2070874
IL-4Rα	I75V	rs1805010
	Q576R	rs1801275
IL-10	−819	rs1800871
	−1082	rs1800896
IL-12B	+159	rs3212227
	+8275	rs2546890
IL-12RB1	−517	rs372889
IL-12RB2	−593	rs1495964
IL-13	−1112	rs1800925
	Q144R	rs20541

[a] National Center for Biotechnology Information, Entrez Gene ID.

2.2. Genotyping

Genomic DNA was extracted from whole blood samples using the QIAamp blood kit (QIAGEN Inc., Chatsworth, CA). Genotyping was performed on genomic DNA using a 5′ nuclease PCR assay. Primers and probes were designed using the Assay-by-Design™ service (PE Applied Biosystems, Foster City, CA). PCR amplification was performed in a volume of 25 μl containing 10 ng genomic DNA, 12.5 μl 2× Taqman® Universal Master Mix, 200 nM of probe, and 900 nM of primer. Cycling conditions were 50 °C for 2 min and 95 °C for 10 min, followed by 50 cycles at 92 °C for 30 sec and 60 °C for 1 min. Amplification was performed using an iCycler® IQ (Biorad Laboratories, Hercules, CA) real-time thermal cycler. All samples with ambiguous results were repeated, as were a random selection of 10% of all samples to ensure laboratory quality control. The selected genes/SNPs are shown in Table 1.

2.3. Microsphere coupling

Pneumococcal polysaccharides (PnPS) were obtained from ATCC, Manassas, VA. Pneumococcal cell wall polysaccharide (CPS) was obtained from Staten Serum Institute (Copenhagen, Denmark). Diphtheria and tetanus toxoids were obtained from University of Massachusetts Biologics Laboratories, Jamaica Plain, MA. The PnPSs were conjugated to spectrally distinguishable microspheres (Luminex, Austin, TX) using 4-(4,6-dimethoxy[1,3,5]triazin-2-yl)-4-methyl-morpholinium [28]. Diphtheria and tetanus toxoids were conjugated to spectrally distinguishable microspheres using 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride and sulfo-*N*-hydroxysuccinimide [28].

2.4. Serum collection and analyses

Blood samples were collected at approximately 1 year of age (mean: 12.5 months). Serum was isolated and stored at −20 °C until analysis. Vaccine-specific antibody responses to PnPSs, diphtheria and tetanus toxoids; and total serum immunoglobulin levels (IgM, IgA, IgG and IgG subclasses) were measured by multiplex assay as previously described [29,30]. Briefly, microspheres coupled to PnPSs, diphtheria and tetanus toxoids were mixed and added to standards and samples diluted in PBS containing 1%BSA, .05%Tween 20, 10 μg/ml of CPS, and 100 μg/ml of PnPS 22. Measurement and data analysis were performed using the Bioplex multiplex testing platform (BioRad, Hercules, CA). Assays were performed in duplicate. Serum levels of IgG, IgA, IgM, and IgG subclasses were measured in duplicate using Beadlyte® assay according to the manufacturer's instructions (Upstate, Lake Placid, NY).

Levels of specific serum antibody to HBsAg were determined using a commercially available enzyme immunoassay according to the manufacturer's instructions (ETI-AB-AUK PLUS, DiaSorin Inc., Stillwater, MN).

2.5. Statistical analyses

SNP-specific deviations from the Hardy–Weinberg Equilibrium (HWE) were tested using chi-squared goodness-of-fit tests. Antibody levels were transformed to their log values before analysis in order for the data to fit the analytic assumptions. Univariate associations between genotypes and log antibody levels were assessed by analysis of variance (ANOVA). General linear models, with adjustments for gender and race, were used to test for differences between antibody levels according to genotypes. Additionally, recessive and dominant models were employed to further investigate the allelic effect with adjustments as described above. The results were not corrected for multiple comparisons since our analyses were based on well defined roles of cytokines in immune responses. The correction for multiple testing would be overly conservative and could inflate the probability of type II error [31,32]. Instead, we reported all tests that reached the 0.05 level of significance with the nominal *p*-values.

3. Results

3.1. Association between vaccine-specific antibody responses and SNPs

SNPs in eleven genes were studied for their influence on vaccine-induced serum antibody levels. All genotype frequencies were in Hardy–Weinberg Equilibrium and were similar to previous reports. After adjusting for gender and race, several significant associations ($p < 0.05$) were found between SNPs in the TNFα, IL-1β, IL-4Rα, IL-10, IL-12B, IL-12RB1, and IL-13 genes and vaccine-specific antibody responses. The TNFα −308 and IL-12B +159 SNPs were associated with variations in median anti-HBsAg antibody levels ($p = 0.003$ and $p = 0.033$, respectively). In addition, the IL-10 −1082 A allele and the IL-4Rα I75V AA genotype were associated with a higher serum antibody levels to diphtheria ($p = 0.039$) and tetanus ($p = 0.037$) vaccinations, respectively (Table 2). Regarding antibody response to PCV7 (Table 3), the IL-4Rα I75V SNP was associated with significant variations in PnPS6B ($p = 0.040$), PnPS14 ($p = 0.039$), PnPS18C ($p = 0.042$), and PnPS23F ($p = 0.044$) serotype specific antibody titers. The TT genotype of IL-10 −819 and IL-13 −1112 SNPs was also associated with lower serum antibody levels to serotypes PnPS6B ($p = 0.027$) and PnPS14 ($p = 0.018$). Furthermore, differences in PnPS9V and PnPS19F serotype-specific antibody titers were associated with the IL-12B1 −517 genotypes ($p = 0.016$ and $p = 0.020$, respectively). None of the other polymorphisms that were examined showed any association with immune responses to vaccine antigens.

3.2. Association between immunoglobulin levels and SNPs

Significant associations ($p < 0.05$) were observed between certain SNPs tested and serum immunoglobulin (IgM, IgA, IgG, and IgG subclasses) levels (Table 4). Total IgG levels varied significantly

Table 2
Significant associations between SNPs in cytokine genes and antibody responses to HBV and DTaP vaccinations.

Vaccine/gene	Position	SNP ID	Genotype	*N* (%)	Median antibody level (IQR)	Adjusted *p*-value[a]
HepB					(IU/l)	
TNFα	−308	rs1800629	GG	109	171.71 (64.5, 549.6)	
			GA	27	176.00 (27.4, 302.70)	
			AA	3	46.6 (9.9, 63.50)	0.003 (GG vs. GA + AA)
IL-12B	+159	rs3212227	CC	76	165.86 (59.1, 388.30)	
			AC	48	147.65 (38.25, 632.75)	
			AA	15	220.9 (74.9, 1104.45)	0.033 (CC vs. AC + AA)
Diphtheria					(IU/ml)	
IL-10	−1082	rs1800896	GG	43	0.30 (0.14, 0.56)	
			GA	71	0.32 (0.19, 0.84)	
			AA	25	0.39 (0.21, 0.96)	0.039 (GG vs. GA + AA)
TetTox					(IU/ml)	
IL-4Rα	I75V	rs1805010	AA	35	0.27 (0.01, 0.65)	
			AG	65	0.16 (0.09, 0.28)	
			GG	39	0.20 (0.08, 0.46)	0.037 (AA vs. AG + GG)

[a] Adjusted for gender and race.

by the IL-1β, IL-2, and IL-4 SNPs. The IL-1β +3953 TT ($p = 0.008$) and the IL-1β −511 ($p = 0.009$) CC genotypes were associated with lower levels of IgG. In addition, the IL-2 −34 TT ($p = 0.030$) and the IL-4 −590 CC genotypes ($p = 0.046$) were associated with lower IgG levels. The TT genotype of IL-10 −819 and IL-2 −34 SNPs were associated with significantly lower levels of IgG1 ($p = 0.021$ and $p = 0.024$, respectively). The IL-1β −511 T allele ($p = 0.040$) and the IL-4Rα I75V AA genotype ($p = 0.001$) were associated with increased levels of IgG2. However, the IL-12B +159 AA and the IL-1β +3953 TT genotypes were related with significantly lower IgG3 levels ($p = 0.014$ and $p = 0.028$, respectively). IgA levels were influenced by the IL-10 −819 ($p = 0.022$) and IL-1β +3953 ($p = 0.002$) SNPs, and IgM levels varied significantly by the IL-10 −819 ($p = 0.036$) and IL-1β −511 ($p = 0.037$) genotypes. There was no correlation between serum Ig levels and vaccine-specific antibody levels.

4. Discussion

Immune responses are inherited as complex quantitative traits and differ in early life due to incomplete maturation of the immune

Table 3
Significant associations between SNPs in cytokine genes and antibody responses to PnPS serotypes.

Serotype/gene	Position	SNP ID	Genotype	*N*	Median antibody level (μg/ml, IQR)	Adjusted *p*-value[a]
PnPS4						
IL-1β	−511	rs16944	CC	67	1.23 (0.68–2.25)	
			CT	58	1.07 (0.55–3.44)	
			TT	14	3.74 (1.65–5.32)	0.019 (CC + CT vs. TT)
PnPS6B						
IL-4Rα	I75V	rs1805010	AA	35	2.76 (1.81, 6.73)	
			AG	65	4.06 (1.81, 8.62)	
			GG	39	2.33 (0.90, 7.82)	0.040 (AA + AG vs. GG)
IL-10	−819	rs1800871	CC	76	2.86 (1.45, 6.59)	
			CT	54	4.20 (1.47,11.43)	
			TT	9	1.75 (1.21, 3.65)	0.027 (CC + CT vs. TT)
PnPS9V						
IL-12RB1	−517	rs372889	CC	35	2.81 (1.61, 3.67)	
			CT	76	3.89 (2.33, 9.22)	0.028 (CC vs. CT)
			TT	28	2.47 (1.21, 8.21)	0.016 (CT vs. TT)
PnPS14						
IL-4Rα	I75V	rs1805010	AA	35	2.26 (0.95, 3.27)	
			AG	65	2.88 (1.27, 5.74)	
			GG	39	1.82 (0.70, 4.58)	0.039 (AA + AG vs. GG)
IL-13	−1112	rs1800925	CC	89	2.26 (0.93, 4.05)	
			CT	46	3.54 (1.36, 8.61)	
			TT	4	0.26 (0.16, 4.15)	0.018 (CC + CT vs. TT)
PnPS18C						
IL-4Rα	I75V	rs1805010	AA	35	2.44 (1.19, 6.25)	
			AG	65	2.17 (1.49, 6.08)	
			GG	39	2.66 (0.74, 4.49)	0.042 (AA + AG vs. GG)
PnPS19F						
IL-12RB1	−517	rs372889	CC	35	1.74 (0.94, 4.01)	
			CT	76	2.66 (1.43, 7.36)	
			TT	28	1.97 (0.89, 3.11)	0.020 (CC vs. CT + TT)
PnPS23F						
IL-4Rα	I75V	rs1805010	AA	35	3.28 (2.32, 10.48)	
			AG	65	5.33 (2.68, 9.34)	
			GG	39	2.66 (1.29, 9.37)	0.044 (AA + AG vs. GG)

[a] Adjusted for gender and race.

Table 4
Significant associations between cytokine SNPs and immunoglobulin levels.

Ig/gene	Position	SNP ID	Genotype	*N*	Median antibody level (mg/dl, IQR)	Adjusted *p*-value[a]
IgG						
IL-1β	+3953	rs1143634	CC	74	675.79 (492.78, 929.98)	
			CT	53	639.07 (530.48, 843.17)	
			TT	12	522.24 (376.78, 613.70)	0.008 (CC + CT vs. TT)
	−511	rs16944	CC	67	565.59 (449.42, 784.55)	
			CT	58	695.49 (565.77, 888.38)	
			TT	14	695.08 (588.77,1004.50)	0.009 (CC vs. CT + TT)
IL-2	−34	rs2069763	GG	63	670.09 (554.86, 917.57)	
			GT	59	639.07 (492.78, 869.00)	
			TT	17	501.86 (404.17, 655.02)	0.030 (GG + GT vs. TT)
IL-4	−590	rs2243250	CC	90	628.59 (482.58, 784.55)	
			CT	42	709.29 (542.38, 978.78)	
			TT	7	648.16 (383.03,1176.13)	0.046 (CC vs. CT + TT)
IgG1						
IL-2	−34	rs2069763	GG	63	643.97 (513.58, 860.70)	
			GT	59	667.13 (468.28, 972.15)	
			TT	17	532.31 (324.74, 706.07)	0.024 (GG + GT vs. TT)
IL-10	−819	rs1800871	CC	76	611.60 (456.40, 838.21)	
			CT	54	740.46 (498.58, 923.81)	
			TT	9	523.95(324.74, 725.84)	0.021 (CC + CT vs. TT)
IgG2						
IL-1β	−511	rs16944	CC	67	56.69 (46.47, 107.43)	
			CT	58	73.03 (49.46, 132.29)	
			TT	14	85.70 (57.90, 167.76)	0.040 (CC vs. CT + TT)
IL-4Rα	I75V	rs1805010	AA	35	111.10 (57.90, 167.76)	
			AG	65	56.69 (45.87, 96.51)	
			GG	39	59.34 (51.58, 105.56)	0.001 (AA vs. AG + GG)
IgG3						
IL-1β	+3953	rs1143634	CC	74	35.15 (24.08, 49.91)	
			CT	53	35.34 (25.71, 57.60)	
			TT	12	24.17 (19.24, 29.63)	0.028 (CC + CT vs. TT)
IL-12B	+159	rs3212227	CC	76	35.63 (23.99, 55.75)	
			AC	48	35.33 (24.80, 51.19)	
			AA	15	29.50 (16.82, 33.72)	0.014 (CC + CA vs. AA)
IgA						
IL-1β	+3953	rs1143634	CC	74	6.35 (4.28, 8.74)	
			CT	53	6.38 (4.34, 9.04)	
			TT	12	3.38 (2.72, 4.20)	0.002 (CC + CT vs. TT)
IL-10	−819	rs1800871	CC	76	5.68 (3.44, 8.26)	
			CT	54	6.45 (4.71, 10.30)	
			TT	9	4.01 (3.23, 6.19)	0.022 (CC vs. CT + TT)
IgM						
IL-1β	−511	rs16944	CC	67	126.73 (93.38, 181.44)	
			CT	58	150.59 (106.15, 199.73)	
			TT	14	145.24 (122.78, 197.38)	0.037 (CC vs. CT + TT)
IL-10	−819	rs1800871	CC	76	137.89 (105.85, 181.08)	
			CT	54	154.79 (107.43, 200.87)	
			TT	9	93.38 (67.30, 126.73)	0.036 (CC + CT vs. TT)

[a] Adjusted for gender and race.

system [33]. Lower antibody responses to vaccine antigens and different immunoglobulin G subclass distribution are known to occur in infants compared to older children or adults [34–37]. Although both Th1 and Th2 type cytokines are involved in specific antibody production in adults, the Th1/Th2 cytokine balance in infancy and early childhood is skewed largely to the Th2 component [38,39]. In this context, IL-4, IL-4Rα, IL-13, and IL-10 are plausible candidate genes to study genetic predisposition to immunologic hypo- or non-responsiveness. Fig. 1 shows the role of selected genes in the development of antibody responses and summarizes the main results of this study. We found IL-4Rα, IL-10, and IL-13 SNPs to be associated with variable responses to HBV, DTaP, and PCV7 vaccinations. Both IL-4 and IL-13 signal through the α-chain of the IL-4 receptor alpha (IL-4Rα) and play a role in regulating antibody responses by B-cells [40]. Recently, the IL-4 −590 T allele which is responsible for increasing transcriptional activity was reported to be associated with higher antibody responses to diphtheria and HBV and lower antibody responses to pneumococcal serotypes [10,23,41]. The IL-13 −1112 T allele has also been shown to alter IL-13 gene expression [42]. The I75V SNP in the IL-4Rα binding region has been associated with increased sensitivity to IL-4 stimulation and was found to be involved in measles vaccine-induced immunity [26]. In our analysis, the IL-4 −590 and IL-13 −1112 SNPs were associated with variations in total IgG level and antibody response against PnPS14 serotype, respectively. On the other hand, the IL-4Rα I75V SNP was associated with the variations in antibody response against tetanus and PnPS serotypes 14, 18C, 23F, and 6B. The I75V SNP was most consistently associated with response to PnPS serotypes. Inconsistent SNP associations across all PnPS serotypes might be related to different serotype immunogenicities and the presence or absence of natural priming [10].

IL-10 is an important immunoregulatory cytokine that plays a major role on antigen presenting cell functions, B-cells growth, and IgG secretion [43]. Both the G allele of IL-10 −1082 SNP and the T allele at position −819 are linked to low IL-10 production [44] and the −819 SNP was found to increase cellular and humoral immunity

to measles vaccination [26]. Antibody titers against pneumococcal proteins were reported to increase in low IL-10 producers [45]. These results were attributed to a balanced Th1/Th2 activation in response to low IL-10 production [26]. We found a significant association between IL-10 −1082 and −819 SNPs and variable antibody responses to diphtheria and PnPS6B vaccines, respectively. Consistent with the role of IL-10 in immunoglobulin production, the IL-10 −819 SNP was associated with variations in serum IgA, IgM, and IgG1 levels [21]. These associations might be related to Th2 polarization during infancy and to the influence of these variations in the regulation of antibody responses.

Although considered a Th2-dependent process, induction of vaccine-specific antibodies require coordinated secretion of pro-inflammatory, Th1 and Th2 cytokines. Pro-inflammatory cytokines IL-1 and TNFα are important regulators of innate immunity [46]. A recent study conducted in a Somali population showed that the rs1799964 SNP in the TNFα gene was associated with lower measles antibody response [47]. We found the TNFα −308 SNP to be associated with variations in the antibody response to HBsAg. This might be related to altered gene product/function or interactions with the genes controlling antigen processing/presentation, such as HLA genes. We have previously demonstrated that individuals possessing an IL-1β +3953 variant, which is associated with higher IL-1β expression, had a significantly higher anti-HBsAg antibody and T-cell lymphoproliferative response following HBV in adults [22]. However, we did not observe a similar association in our study population of infants although the IL-1 β +3953 variant was associated with variations in serum IgG, IgA, and IgG3 levels. Different findings might be related to delayed maturation of the Th1 functions and influence of other factors such as maternal antibodies and nutrition in infancy. It is also possible that environmental and lifestyle factors (e.g., smoking, inhaled antigens, UV exposure) may influence immune responsiveness in adults.

The other Th1 cytokines, IL-12 and IL-2, are also important components of immune response regulation. The heterodimeric cytokine IL-12 is crucial for Th1 polarization and optimal antigen presenting cell function. In the case of HBV, IL-12 levels were highly elevated in the high responders and demonstrated a positive correlation with antibody titers [48]. We found the IL-12B +159 AA genotype to be associated with higher antibody response to HBsAg. Previous studies have reported that the −517C SNP in the IL-12RB1 subunit was associated with low antibody and low lymphoproliferative responses to measles and mumps vaccinations, respectively [26,49]. Our study demonstrated that the −517C SNP was associated with variable immune responses to PnPS9V and 19F serotypes. IL-2 also induces the proliferation and differentiation of T- and B-cells and promotes the induction of immunoglobulin secretion [50]. While the −34G variant of the IL-2 gene was associated with higher antibody and lymphoproliferative responses to measles vaccinations [26], the −330G variant was reported to be associated with the responder phenotype in HBV vaccination [23]. We found an association between the IL-2 −34G allele and higher total IgG and IgG1 levels.Genetic association studies have reported inconsistent findings on associations between immune response gene variations and vaccine-specific responses. It is possible that genetic factors may have differential effects on immune response based on antigenic stimulus, interactions between pathogen and host, and gene–gene interactions [26]. Immune response elicited by a vaccine is the cumulative result of interactions driven by genes in immune-response network. This network includes genes necessary for activation/suppression of immune responses, their variations and interactions, epigenetic effects, and other host response factors [51]. Since cytokines are major components of this network, the majority of the SNPs analyzed in the present study might not only directly influence the regulation of their own genes, but might be involved in the dynamic/complex relations of other cytokines through their own genes. For example, variants of IL-4Rα have shown to influence secretion of IL-4, IL-10, and IL-12 [51].

Together, our findings support the significance of genetic variation in directly or indirectly interacting cytokine genes in antigen-specific immune responses, which in turn, can influence vaccine responsiveness. It is possible that the effect of each polymorphism on its own might be limited and could be attributable to another co-inherited variant or to the link with HLA alleles; however, based on previous studies and the role of selected cytokines in immune response network, it is most likely that our findings are associated with the variations studied. Prospective studies in a larger validation population will be needed to confirm these results. A better understanding of genetic factors that determine an effective response is important for more uniformly effective vaccine design, improved vaccine immunogenicity, and personalized vaccine development.

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