Original article

Parental smoking impairs vaccine responses in children with atopic genotypes

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Background: Gene-environment interactions play central roles in controlling postnatal maturation of immune function, but their effects on infant vaccine responses are unknown. Genetic variants associated with atopy and the environmental factor of exposure to parental smoking (PS) of tobacco independently alter immune responses.

Objective: We sought to investigate the hypothesis that genetic variants associated with atopy and their interaction with PS influence infant vaccine responsiveness.

Methods: In 200 infants with parental atopic history, relationships were sought between polymorphisms in the IL-4, IL-4 receptor α (IL-4Rα), and IL-13 genes; PS; and immune responses to diphtheria/tetanus vaccination.

Results: Analyses stratified by PS unmasked negative associations between atopic alleles of these genes and vaccine outcomes. The most consistent involved the IL-4Rα 551 QR/QQ genotypes, which were associated with reduced IgG levels ($P = .02$) and T-cell responses (IFN-γ, $P = .002$; IL-10, $P = .01$; 1L-13, $P = .01$; IL-5, $P = .06$) to tetanus toxoid and parallel reductions in polyclonal T-cell responses and innate immune responses in PS-exposed infants.

Conclusion: PS potentiates suppressive effects of variants in immune response genes in children. These effects are not observed in the absence of this exposure. Ultimately, this finding might have implications for infant vaccination in countries with high smoking rates. It might also have broader implications in relation to environmental toxicology because it demonstrates specific mechanisms through which the developing immune system might be differentially sensitive to low-level toxicant exposures.

Clinical implications: PS interacts with genes associated with atopy to impair vaccine responses. These interactions might have vaccine design and public health implications. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

Key words: *Genetics, environmental exposure, atopy, public health, smoking, toxicology*

Globally, more than 50% of children less than 5 years of age who die do so from infectious diseases.[1] Vaccination is unequivocally important in the prevention of these diseases. The importance of genetic influences on vaccine responses is suggested by differences in vaccine responses between individuals and ethnic groups[2] and evidence for high heritability of specific antibody and T_H2 cytokine responses to vaccines.[3-6] Understanding these genetic influences should elucidate vaccine response mechanisms, ultimately resulting in improved vaccine efficacy.

Associations between specific genes and vaccine responses have been reported,[2,6] but studies have predominantly focused on HLA and Ig allotype genes.[7,8] Many other genes might modulate vaccination responses, and therefore a targeted approach is necessary. Genes associated with atopy are potentially important because an atopic family history and atopic eczema are associated with delayed maturation of responses to tetanus[9] and pneumococcal vaccine, respectively.[10] Therefore variations in genes previously associated with atopy, including IL-4, IL-4 receptor α (IL-4Rα), and IL-13,[11] might influence vaccine responses. IL-4 and IL-13 are pleiotropic cytokines that interact with IL-4Rα to signal to effector cells.[12] Their functions include induction of IgG4 and IgE antibody synthesis by B lymphocytes and differentiation to a T_H2 phenotype.

Environmental factors also influence vaccine responses. Notably, smoking has been associated with both impaired[13,14] and increased responses to vaccination.[15] Therefore because exposures in early life are likely to interact with a child's genotype to modulate response to environmental factors[16] and the probability of detecting genetic effects is increased when analyses are stratified by specific environmental factors, including environmental tobacco smoke (ETS),[17] we investigated the effects and interaction of single nucleotide polymorphisms (SNPs) in the IL-4, IL-13, and IL-4Rα genes and the environmental factor parental smoking (PS) on responses to diphtheria and tetanus vaccine in a cohort of children at risk of atopy. We hypothesized that genetic variants

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Abbreviations used
DTPa: Diphtheria-tetanus-pertussis acellular
DTPw: Diphtheria-tetanus-pertussis whole cell
ETS: Environmental tobacco smoke
IL-4Rα: IL-4 receptor α
PS: Parental smoking
SEB: Staphylococcal enterotoxin B
SNP: Single nucleotide polymorphism
SPT: Skin prick test
TLR: Toll-like receptor

associated with atopy would modulate vaccine responses and that these effects would be modified by PS.

METHODS

Population

Two hundred children aged 2 years, born between July 1996 and July 1998, from a previously described Western Australian birth cohort were recruited on the basis of parental history of atopy.[18] PS was defined by parental report questionnaires, when children were 2 years old, of current cigarette smoking by either or both parents. Skin prick test (SPT) reactivity to a standard panel of allergens (Hollister Stier Labs, Spokane, Wash), which included house dust mite (*Dermatophagoides pteronyssinus*), perennial rye grass, cat pelt, *Alternaria tenuis*, *Aspergillus fumigatus*, fresh cow's milk, and fresh hen's egg. Histamine and normal saline were included as positive and negative controls, respectively, as previously described.[18] An SPT response was regarded as positive if the wheal diameter was 3 mm or larger. Data on other potentially important exposures were collected, including the dichotomous variables day-care attendance, pet exposure (dog, cat, or both), and other children in the house.

Vaccines and vaccination schedules

At ages 2, 4, and 6 months, the subjects received acellular pertussis (DTPa; Infanrix; GlaxoSmithKline, Belgium) or whole-cell pertussis (DTPw; Triple Antigen; CSL, Australia), oral polio (Polio Sabin; GlaxoSmithKline), and intramuscular *Haemophilus influenzae* type b (HibTITER; Wyeth-Ayers, Madison, NJ) vaccines. A measles-mumps-rubella vaccine (MMRII; Merck, Sharp & Dohme, Whitehouse Station, NJ; or Priorix; GlaxoSmithKline) was administered at age 12 months. At 18 months, a further dose of diphtheria-tetanus-pertussis acellular (DTPa) or DTP whole cell (DTPw) and *Haemophilus influenzae* type b vaccination was given. The last DTP vaccine before blood collection was DTPa in 44.5% of individuals. Three percent of individuals never received DTPw before blood collection.

Measurement of vaccine-specific antibody concentrations

Blood samples were obtained at 2 years of age (mean, 2 years and 15 days; range, 1 year and 335 days to 2 years and 35 days), and serum was isolated and stored at −20°C. The levels of anti-diphtheria and anti-tetanus IgG were measured by means of ELISA.

Cell preparation and culture

Peripheral blood was collected at age 2 years and processed to yield PBMCs, as previously described.

PBMCs were cultured at a concentration of 1×10^6 cells/mL in serum-supplemented RPMI-1640 for stimulation with tetanus toxoid or Toll-like receptor (TLR) ligands or in AIM-V serum-free medium (Gibco Life Technologies, Grad Island, NY) supplemented with 4×10^{-5} mol/L 2-mercaptoethanol for stimulation with the polyclonal T-cell mitogens PHA and staphylococcal enterotoxin B (SEB). Cells were incubated either alone or together with tetanus toxoid (0.5 LF/mL; CSL), PHA (1 μg/mL; Murex Biotech), SEB (200 ng/mL; Sigma), or the TLR ligands lipoteichoic acid (2 μg/mL; Invivogen, San Diego, Calif), pansorbin (0.01%; Calbiochem, San Diego, California), or the combination of bacterial LPS (1 ng/mL; Alexis Biochemicals, San Diego, Calif) with IFN-γ (10 ng/mL; PharMingen, San Diego, Calif).

Time-resolved fluorometry of cytokines. As described elsewhere,[9] PBMCs were cryopreserved at collection and batch analyzed in groups of 10 within a short period and with identical reagents. The levels of IL-5, IL-10, IL-13, and IFN-γ in culture supernatants was determined by using in-house time-resolved fluorometry, as previously described.[19] The limit of detection for these assays was 10 pg/mL. As previously described,[20] responses that were less than the limits of detection (including zeros) were ascribed a value equivalent to half the limit of detection. Limitations in cell numbers caused by small-volume blood samples in some individuals resulted in fewer cytokine response outcomes compared with antibody response outcomes.

Genotyping. Genotyping was performed by using PCR-RFLP (IL-4 C-589T, IL-4 G2979T, IL4-Rα Q551R, and IL-13 C-1112T) and denaturing HPLC (IL-4Rα I50V and IL-13 R130Q) in an automated system (Varian Helix-System) according to the method developed by Oefner and Underhill.[21] Accession number, forward and reverse primer sequences, and restriction enzymes, where relevant, for each SNP are described below. Underlined bases in primers indicate a base change to create restriction enzyme site and amino acids are numbered from the beginning of the mature protein.

- IL-4 C-589T: rs2243250, forward primer ACTAGGCCTCACCTG ATACG, reverse primer GTTGTAATGCAGTCCTCCTG, *Bsm*FI.
- IL-4 G2979T: rs2227284, forward primer TAGGTCCTGGGCTT-CACAG, reverse primer TTAGCTCTCTTTGGTAAATAGGGA A, *Hin*fI.
- IL-4Rα Ile50Val: rs1805010, forward primer GCAAGAGAGGC AACCCTA, reverse primer GCCTCCGTTGTTCTCAG.
- IL-4Rα Gln551Arg: rs1801275, forward primer GCCCCCGTCT CGGCCCCCACCAGTGGCTACC, reverse primer GCCCCAAA CCCACATTTCTCTGG, *Msp*I.
- IL-13 C-1112T: rs1800925, forward primer CGAGGACAGGA CGGAGGGAGCCT, reverse primer GTCGCCTTTTCCTGCTC TTCCCG, *Bst*UI.
- IL-13 Arg130Gln: rs20541, forward primer CTTCCGTGAGGAC TGAATGAGACAGTC, reverse primer GCAAATAATGATGCT TTCGAAGTTTCAGTGGA.

Statistics

Responses were skewed and therefore were log transformed to approximate a normal distribution, as previously described.[20] Regression models were used to assess genetic associations. The time between last vaccine dose and blood sampling was negatively correlated with vaccine responses (diphtheria toxoid, $P < .0005$; tetanus toxoid, $P < .0005$) and IL-10 responses ($P = .049$) and was therefore included in the models as a confounding variable. Subsequently, PS was added to these models, and then the interaction effect of genotype and PS was determined. Given, in general, the low frequency of alleles previously associated with atopy, models dominant for these alleles (IL-4 −589T,[21] IL-4 2979T,[22] IL-4Rα I50,[23] IL-4Rα 551R,[24] IL-13 −1112T,[25] and IL-13 130Q[26]) were analyzed. Nonparametric analyses were also performed and were in accordance with findings from parametric analyses. Interactions were investigated by comparison of geometric means corrected for time between last DTP vaccine dose and blood sample collection between genotype groups after stratification for PS. A P value of less than .05 was

TABLE I. Patient and phenotypic characteristics with geometric mean IgG levels and 95% CIs for diphtheria and tetanus toxoids and tetanus toxoid–stimulated cytokine levels stratified by PS

	No PS (n = 158)			PS (n = 42)			
	n	%		n	%		*P* value
Male sex	97	61		19	45		.07
SPT ≥3 mm	35	22		10	23		.78
	n	**GM**	**95% CI**	**n**	**GM**	**95% CI**	
IgG (IU/mL)							
DiphTox	158	1.23	1.1-1.5	42	1.06	0.7-1.6	.50
TetTox	158	2.09	1.8-2.5	42	1.84	1.3-2.7	.51
TetTox-stimulated cytokine (pg/mL)							
IFN-γ	125	108.0	71-164	36	124.0	64.5-238	.75
IL-5	125	142.4	101-200	35	84.5	44.9-159	.15
IL-10	125	8.7	6.6-11.3	36	7.1	4.5-11.0	.46
IL-13	125	410.0	304-554	36	248.9	133-465	.13

Vaccine responses are corrected for time between last dose of vaccine and blood sampling.
GM, Geometric mean; *DiphTox*, diphtheria toxoid; *TetTox*, tetanus toxoid.

considered significant. No adjustments made for multiple testing in investigations were based on an *a priori* hypothesis, and we have described all tests performed.[22,23] All analyses were performed with SPSS version 11.5 software (SPSS, Inc, Chicago, Ill).

RESULTS

Phenotypic characteristics and associations

Overall, 21% (n = 42) of the cohort was exposed to PS. There was no significant association between PS, sex, or SPT results and responses; between genotype and SPT results; or between the combination of genotype and smoke exposure and SPT results. Furthermore, there was no significant interaction effect of atopy (SPT result) and PS on any of the responses studied. Geometric means and 95% CIs of the total specific IgG levels to diphtheria and tetanus toxoids stratified by PS are shown in Table I. There was no significant relationship between type of DTP vaccine given and vaccine responses (data not shown). Correlations between antibody and cytokine responses were investigated by using Spearman rank correlation. All significant correlations were positive. These included tetanus and diphtheria antibody responses ($r = .599$, $P < .000$), tetanus antibody responses and all tetanus toxoid–stimulated cytokine responses ($r = 0.274$ to 0.371; $P = .0001$ to $P < .000$), tetanus antibody responses and SEB-stimulated IFN-γ responses ($r = 0.191$, $P = .011$), and pansorbin-stimulated IL-10 responses ($r = 0.187$, $P = .03$). No diphtheria antibody vaccine response was less than 0.01 IU/mL, the generally accepted minimum level giving some degree of protection,[24] and 1.4% of tetanus antibody levels were less than the protective level of 0.15 IU/mL.[25]

Investigation of genotypic associations and the interaction of smoking and genotype on vaccine-specific immune responses

Genotypic effects. All polymorphisms were in Hardy-Weinberg equilibrium, and allele frequencies were consistent with previous reports (see Table E1 in the Online Repository at www.jacionline.org). Analysis of the IL-4 C-589T SNP revealed a positive association of the T allele with antibody response to diphtheria toxoid ($P = .049$ and $P = .02$, respectively; Fig 1). There were no further significant associations between the remaining loci and vaccine responses.

Genotypic effects after stratification by PS. Vaccine responses were compared between genotype groups within those exposed and unexposed to PS to investigate whether the relation of genotype to outcome differed depending on PS exposure.

In unexposed subjects those with IL-4 −589 CT/TT genotypes had higher diphtheria ($P = .005$) and tetanus ($P = .04$) antibody responses (Table II) compared with those with subjects with the CC genotype. Furthermore, those with IL-4Rα I5OV II/IV genotypes had nonsignificantly higher diphtheria ($P = .08$) antibody responses compared with those with the VV genotype.

In exposed subjects those with the IL-4 −589 CT/TT genotypes, compared with those with the CC genotype, had decreased tetanus antibody responses ($P = .02$) and nonsignificantly decreased tetanus toxoid–stimulated IL-10 ($P = .08$) responses. Additionally, those with IL-4Rα Q551R QR/RR genotypes, compared with those with the QQ genotype, had reduced antibody responses (diphtheria, $P = .03$; tetanus, $P = .02$; see Fig 2) and tetanus toxoid–stimulated IFN-γ ($P = .002$), IL-10 ($P = .014$), and IL-13 ($P = .01$) responses (see Table II and Figs 2 and 3). There was also a nonsignificant reduction in tetanus toxoid–stimulated IL-5 ($P = .06$) responses. Furthermore, those with the IL-13 −1112 CT/TT genotypes had lower tetanus toxoid–stimulated IL-5 ($P = .002$) and IL-10 ($P = .02$) responses compared with those with the CC genotype. Significant findings in exposed individuals were reexamined, with adjustment for other potentially important exposure variables (ie, day-care attendance, pet exposure, and number of siblings); the results were largely unchanged.

Interaction effects. Regression models were used to explore the possibility of an interaction between genotype

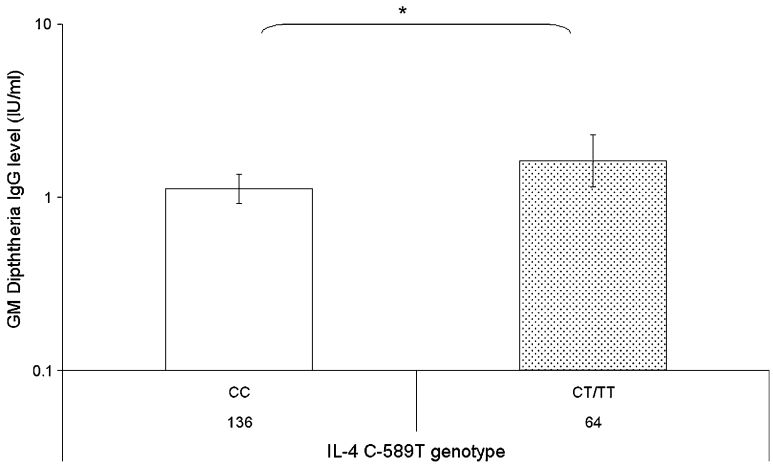

FIG 1. Specific IgG responses to diphtheria toxoid categorized by IL-4 C-589T genotype (*$P < .05$). Log geometric mean *(GM)* and 95% CI are plotted. *Numbers on the horizontal axis* are the numbers of individuals within each genotype group. The *gray bar* represents atopic genotypes. Values plotted are corrected for time between last dose of vaccine and blood collection.

and PS on vaccination responses. The interaction effects of IL-4 C-589T genotype and PS on antibody responses to diphtheria and tetanus antibody responses were significant ($P = .02$ and $P = .002$, respectively). This effect was also significant for the IL-4Rα I50V and tetanus antibody responses ($P = .02$). Other smoking-genotype interaction effects approached significance. These included IL-4 C-589T/tetanus toxoid-stimulated IL-5 responses ($P = .06$), IL-4 G2979T/tetanus antibody responses ($P = .06$), IL4Rα I50V/diphtheria antibody responses ($P = .06$), and IL-4Rα Q551R diphtheria ($P = .08$) and tetanus ($P = .07$) antibody responses (see Table II). Significant associations were reexamined with the inclusion of other exposure variables, as above, in the linear regression models; results were largely unchanged.

Investigation of polyclonal T-cell and innate immune responses

The most consistent finding was the interaction of IL-4Rα Q551R and PS on vaccine responses, including specific IgG production by B lymphocytes. This interaction was further explored in cell populations that exert their effects upstream of IgG-producing B lymphocytes, notably T_H cells, which drive Ig class switching in B lymphocytes, and cells of the innate immune system, which activate T_H cells, to investigate the site or sites of action of this polymorphism in the immunologic cascade leading to IgG production. Hence in addition to cytokine responses to vaccine-specific stimuli, cytokine responses to polyclonal T-cell activation stimuli and to TLR ligands that trigger innate immune cell responses were determined and analyzed after stratification for PS exposure. There were no significant genotype-related differences among those not exposed to PS; however, in PS-exposed subjects differences were demonstrated. Specifically, and consistent with analyses of vaccine-specific responses, IL-4Rα 551 QR/RR genotypes were associated with lower responses compared with the QQ genotype (Figs 3 and 4). Significant differences in cytokine responses to polyclonal stimuli included PHA-induced IL-10 ($P = .04$), SEB-induced IL-10 ($P = .02$), and PHA-induced IFN-γ ($P = .02$, Fig 4). Significant differences in TLR ligand–triggered innate responses observed included pansorbin-induced IL-10 ($P = .02$), IFN-γ ($P = .03$), and TNF-α ($P = .03$) and lipoteichoic acid–induced IL-10 ($P = .03$), IFN-γ ($P = .04$), and TNF-α ($P = .048$; Fig 5 and see Table E2 in the Online Repository at www.jacionline.org).

DISCUSSION

This study demonstrated genetic influences on immune responses to diphtheria and tetanus vaccination. Individuals with IL-4 −589 CT/TT genotypes (ie, genotypes containing at least one allele previously associated with atopy) had an increased IgG antibody response to diphtheria vaccination. Consistent with our findings, the IL-4 −589T allele was associated with increased antibody responses against malaria antigens,[26] and IL-4 haplotypes containing the IL-4 −589T allele were found more commonly in hepatitis B vaccine responders compared with nonresponders.[27] It is also pertinent to note studies on vaccine responses in children, including those to diphtheria and tetanus vaccines, revealed a major contribution to genetic variance from genes outside HLA loci,[6] and in this context our current results point to genes in the IL-4/IL-13 axis.

Prima facie, the finding of increased antibody responses among children with IL-4 alleles associated with atopy contrasts with reports of delayed maturation of adaptive immune competence in children at high genetic risk of atopy (including those with a parental history of atopy), in particular in relation to the functions of T_H cells, which drive antibody production.[28] However, we have shown that attenuation of T_H cell activity is transient

TABLE II. Association of genotype with diphtheria and tetanus toxoid IgG responses and tetanus toxoid–stimulated cytokine responses, interaction effect of genotype and PS on these responses, and effect of stratification of genotype by PS exposure

Locus and phenotype	Main effect of genotype *P* value	Interaction effect *P* value	No PS (n = 158)		PS (n = 42)	
			Genotype effect	*P* value	Genotype effect	*P* value
IL-4 C-589T						
DiphTox	**.049**	**.02**	CT, TT ↑	**.005**	Nil	.40
TetTox	.60	**.002**	CT, TT ↑	**.04**	CT, TT ↓	**.02**
IFN-γ	.51	.33	Nil	.30	Nil	.34
IL-5	.12	*.06*	Nil	.23	Nil	.42
IL-10	.77	.23	Nil	.59	CT, TT ↓	*.08*
IL-13	.83	.98	Nil	.94	Nil	.98
IL-4 G2979T						
DiphTox	.88	.35	Nil	.50	Nil	.43
TetTox	.53	*.06*	Nil	.80	GT/TT ↓	*.06*
IFN-γ	.20	.12	Nil	.27	Nil	.39
IL-5	.29	.31	Nil	.33	Nil	.36
IL-10	.33	.11	Nil	.25	Nil	.62
IL-13	.96	.65	Nil	.62	Nil	.42
IL-4RA I50V						
DiphTox	.77	*.06*	II, IV ↑	.08	Nil	.24
TetTox	.34	**.02**	Nil	.12	Nil	.14
IFN-γ	.50	.65	Nil	.24	Nil	.11
IL-5	.93	.26	Nil	.56	Nil	.44
IL-10	.60	.31	Nil	.35	Nil	.19
IL-13	.45	.10	Nil	.56	Nil	.74
IL-4RA Q551R						
DiphTox	.33	*.08*	Nil	.76	QR, RR ↓	**.03**
TetTox	.42	*.07*	Nil	.85	QR, RR ↓	**.02**
IFN-γ	.50	.19	Nil	.97	QR, RR ↓	**.002**
IL-5	.24	.99	Nil	.57	QR, RR ↓	*.061*
IL-10	.18	.42	Nil	.58	QR, RR ↓	**.01**
IL-13	.64	.21	Nil	.55	QR, RR ↓	**.01**
IL-13 C-1112T						
DiphTox	.34	.72	Nil	.59	Nil	.53
TetTox	.12	.55	Nil	.38	Nil	.17
IFN-γ	.25	.69	Nil	.32	CT, TT ↓	**.002**
IL-5	.55	.42	Nil	.82	Nil	.173
IL-10	.62	.18	Nil	.51	CT, TT ↓	**.02**
IL-13	.33	.31	Nil	.12	Nil	.301
IL-13 R130Q						
DiphTox	.84	.93	Nil	.62	Nil	.85
TetTox	.78	.68	Nil	.92	Nil	.26
IFN-γ	.39	*.09*	Nil	.69	Nil	.14
IL-5	.55	.23	Nil	.49	Nil	.98
IL-10	.61	.24	Nil	.87	Nil	.92
IL-13	.33	.27	Nil	.22	Nil	.42

P values of less than .05 are shown in bold. *P* values in italics are those approaching significance. *Arrows* indicate an increase (↑) or decrease (↓) in IgG or cytokine levels compared with those of the remaining genotypes at each locus.

in children at high genetic risk of atopy and rebounds at the end of infancy to create a state of heightened activity relative to that seen in children at low risk.[9] Further indicative of this rebound effect are the heightened vaccine-specific T_H memory responses in 6-year-old atopic children.[28] Collectively, these observations suggest that the direction and magnitude of the genetic effects associated with atopy risk on vaccine responses in childhood vary with age. In this context it is noteworthy that age-related variation in effect has been shown with another polymorphism associated with atopy, namely CD14 C−159T.[29]

Our most novel finding concerned interactions between genotype and PS on antibody responses to vaccine. Specifically, atopy-associated SNPs in the genes for IL-4 and IL-4Rα were associated with increased vaccine responses in children of nonsmoking parents but with lowered responses in children of smokers. Further support for the notion that environmental factors interact with genes in the IL-4/IL-13 axis to modulate immune responses in

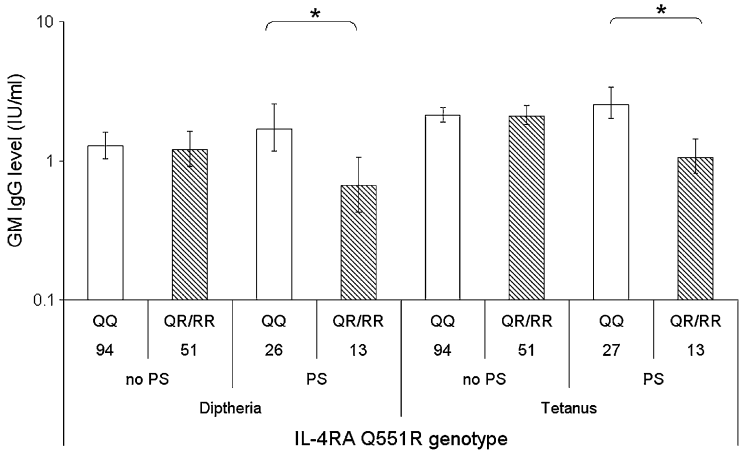

FIG 2. Specific IgG responses to diphtheria and tetanus toxoids compared between IL-4Rα Q551R genotypes in subjects exposed *(PS)* and unexposed *(no PS)* to PS (*$P < .05$*). Log geometric mean *(GM)* and 95% CI are plotted. *Numbers on the horizontal axis* are the number of individuals within each genotype group. *Gray bars* represent atopic genotypes. Values plotted are corrected for time between last dose of vaccine and blood collection.

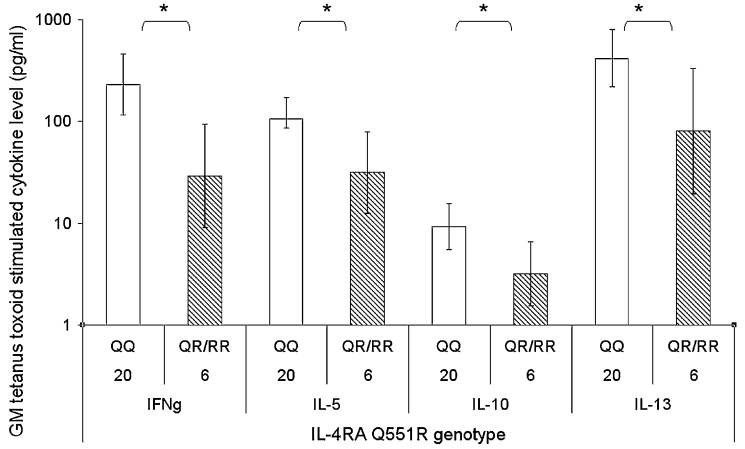

FIG 3. Tetanus toxoid–stimulated cytokine responses compared between IL-4Rα Q551R genotypes in subjects exposed to PS (*$P < .05$*). Log geometric mean *(GM)* and 95% CI are plotted. *Numbers on the horizontal axis* are the numbers of individuals within each genotype group. *Gray bars* represent atopic genotypes. Values plotted are corrected for time between last dose of vaccine and blood collection.

childhood are the day-care/gene (IL-4Rα) interaction effect on IFN-γ responses in the first year of life[16]; the association of the IL4Rα Q576R polymorphism in children with atopic eczema not given antibiotics, although not in those given antibiotics[30]; and linkage studies implicating ETS in the unmasking of sensitivity to asthma susceptibility genes.[11,17] This study identifies specific genetic targets for ETS effects on responses to vaccine antigens. Moreover, that the effects in this study are only evident against a specific genetic background further supports the gene-environment paradigm in determining phenotype.

IL-4 and IL-4Rα are highly plausible candidate genes in the context of this study, given the major role of IL-4 in driving the growth and differentiation of the B lymphocytes ultimately responsible for the magnitude of the primary outcome, namely antibody production. However, the antibody data *per se* provide no insight into the nature of or sites of action of the ETS effects that unmask susceptibility to these genetic variants. To investigate this, we assessed the activity of other cell types that function in antibody responses upstream of B lymphocytes. Specifically, we examined T_H memory cells, which are the principal sources of the cytokines driving B-lymphocyte maturation, and cells of the innate immune system, which provide the initial signals to trigger T_H cell activation in response to vaccine antigens or polyclonal stimuli. In children with the IL-4Rα Q576 QR/RR genotype, a broad spectrum of vaccine-specific (Figs 2 and 3) and polyclonal (Fig 4) cytokine responses were attenuated in parallel with specific IgG antibody production, although only

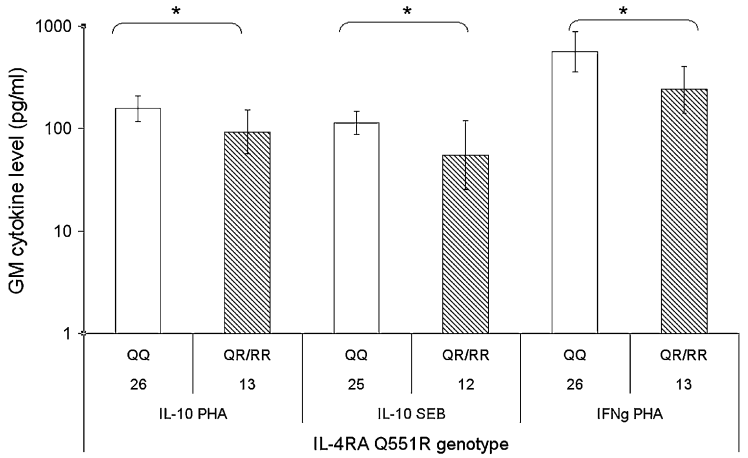

FIG 4. PHA- and SEB-induced cytokine responses compared between IL-4Rα Q551R genotypes in subjects exposed to PS (*$P < .05$). Log geometric mean *(GM)* and 95% CI are plotted. *Gray bars* represent atopic genotypes. *Numbers on the horizontal axis* are the numbers of individuals within each genotype group.

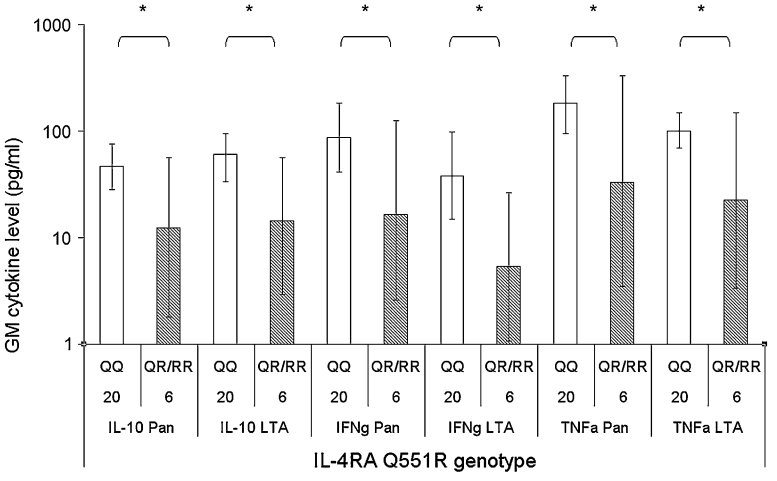

FIG 5. Lipoteichoic acid (LTA) and pansorbin-induced (Pan) cytokine responses compared between IL-4Rα Q551R genotypes in subjects exposed to PS (*$P < .05$). Log geometric mean and 95% CI are plotted. *Gray bars* represent atopic genotypes. *Numbers on the horizontal axis* are the numbers of individuals within each genotype group.

in those with ETS exposure. Of particular note, the capacity of cells of the innate immune system in ETS-exposed children with the IL-4Rα Q576R QR/RR genotype to respond to the TLR ligands lipoteichoic acid and pansorbin was also markedly attenuated (Fig 5).

Cells of the innate immune system are widely distributed on and within mucosal tissues interfacing with the outside environment and provide the first line of immunologic defense against foreign antigens, as well as playing an instructive role in controlling ensuing adaptive immunity. Accordingly, our finding of defective TLR responses in these children pinpoints the ETS effects on vaccine immunity to this initial cellular step in the immunologic cascade. This finding is highly plausible in light of earlier studies. In particular, active smoking has been demonstrated to markedly attenuate priming of immunologically naive adults against influenza vaccine,[13] and passive smoke exposure of animals has been shown to exert comparable effects.[31] Of particular note, these earlier studies identified cells of the innate immune system as one of the primary targets for the immunomodulatory effects of tobacco smoke.[32] Moreover, in animal models these ETS effects could be reproduced by vapor-phase components, including oxides of nitrogen, which are common constituents of urban air pollution,[33] suggesting that these findings might be generalizable to environmental factors other than ETS.

Precisely how ETS exposure of children influences adaptive immune function outside the respiratory tract remains to be determined. However, given that ETS exposure can deleteriously affect immune functions in adults, our present results might not be surprising. The molecular mechanism or mechanisms mediating ETS effects on the specific genes is unknown. However, it is

clear that they do not involve ETS-mediated changes in DNA sequence and are hence epigenetic. There are numerous reports of epigenetic regulation of immune response genes, including IL-4,[34,35] and epigenetic effects of smoking on immune function have been reported in other systems, but epigenetic mechanisms were not directly addressed here. Smoke exposure has been implicated in experimental studies in damage to both respiratory mucosal and systemic immune function[31-33] and in this case might work through either route. That stimulation of distal mucosal surfaces by environmental agents can modulate the differentiation, functional activity, or both of bone marrow–derived immune cells is well established, the archetypal example being stimulation of the gastric mucosa by commensal bacteria, which is recognized as a principal mechanism driving the postnatal maturation of the immune system during infancy.[36] The corollary that signals generated from the respiratory mucosa by environmental stimuli, including ETS, can contribute to (and possibly interfere with) this process is accordingly not radical.

Given that the vast majority of antibody responses were greater than generally accepted minimum levels of protection for diphtheria and tetanus infection and that cytokine responses were determined *in vitro*, the clinical implications of our findings are unknown. However, in interpreting these responses, it is notable that no antibody level can confer absolute protection.[24] Additionally, the cytokine responses were also impaired, and the clinical implication of this in combination with the reduced antibody levels is also unknown. If our findings are to have health implications, these might be greatest in developing countries because cigarette consumption is increasing in developing nations,[37] and the alleles associated with impaired immune responses in children of smokers are more common in many of these countries.[38,39] Further research is needed to clarify these issues.

This study has several limitations, including the following. First, we were unable to assess the differential effects of maternal and paternal smoking, *in utero* versus *ex utero* exposure, or dose-dependent effects because of the relatively small number of smoking mothers. Second, cotinine levels were not measured, and using parental reports to assess smoking exposure is subject to recall bias. However, such bias is likely to be independent of the genotypes studied and would therefore likely skew the estimates of interaction effects toward the null hypothesis.[40] Third, without a healthy control group, it cannot be said whether the demonstrated associations are specific to or independent of a high genetic risk of atopy. Finally, no corrections were made for multiple testing. We elected not to make such corrections because (1) they can be overly conservative and might inflate type II errors and (2) our analyses were based on an *a priori* hypothesis.[22] Instead, we have chosen to describe all statistical analyses carried out and to discuss the results. We agree with Rothman[23] that there is little to be gained by adjustment, provided that all the analyses are described when the study is reported, and there is a lot to be lost by it if anything other than the results of all hypothesis testing are reported.

In conclusion, this study identifies genetic influences on and gene-smoking interactions affecting vaccine responses. It suggests a role of genetic variants associated with atopy in modulation of vaccine responses and supports the joint consideration of genetic and environmental factors in studies of complex phenotypes. Accordingly, failure to account for gene-environment interactions could have contributed to inconsistent associations of both genetic and environmental factors with vaccination responses. Understanding these interactions might enhance understanding of mechanisms regulating vaccine responses. Finally, and particularly because effects on multiple arms of the immune response were demonstrated, these findings might have implications for the pathogenesis of immune-mediated disorders.

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