

Exposure to a farming environment has allergen-specific protective effects on T_H2-dependent isotype switching in response to common inhalants

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Background: IgE synthesis by human B cells results from allergen-dependent, T_H2-mediated isotype switching. Exposure to a farming environment protects against IgE responses.

Objective: We reconstructed allergen-dependent switching patterns *in vivo* to identify the level or levels at which farm exposure acts to protect against atopy.

Methods: Serum IgG1 to IgG4 and IgE to grass (rPhl p 1 and rPhl p 5), cat (rFel d 1), and mite (rDer p 2) were assessed by means of ELISA in the Allergy and Endotoxin study population (812 children). Farm exposure was defined as currently living on a farm, exposure to stables/farm milk in the first year of life, or both.

Results: Farm exposure did not affect allergen-specific IgG2 and IgG3 levels but had complex allergen-specific effects on

IgG1, IgG4, and IgE levels. Exposure protected against grass-specific responses at every step along the IgG1/IgG4/IgE switching pathway but had no significant effect on mite responses. Protection from cat responses was concentrated at the IgG1 level. For all allergens, failure to express IgG1 was associated with low prevalence of IgG4 or IgE responses. Notably, coexpression of IgG1, IgG4, and IgE to grass was associated with increased risk of allergic disease and higher IgE levels compared with production of IgG1 and IgE without IgG4, suggesting IgG4 coexpression marks stronger activation of T_H2-dependent events.

Conclusion: The protective effects of farm exposure were confined to T_H2-dependent IgG1, IgG4, and IgE expression and were allergen and switch stage specific, suggesting that distinct mechanisms regulate individual steps within allergen-induced class switching *in vivo*.

Clinical implications: Environmental interventions to prevent IgE expression might need to be tailored to specific allergens. (J Allergy Clin Immunol 2006;117:1001-1013.)

Key words: Asthma, IgE, IgG, immunoglobulin switch region, farm exposure, immediate hypersensitivity, allergy, atopy

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IgE and IgE-mediated events play a major effector role in both acute and chronic allergic inflammation. Antigen-mediated cross-linking of IgE bound to FcεRI results in mast cell and basophil degranulation and release of mediators critical for immediate hypersensitivity reactions.¹ Furthermore, IgE-mediated engagement of FcεRI upregulates the expression of cytokines and chemokines, enhances mast cell survival, and promotes T-cell activation, further amplifying the inflammatory process, in combination with IgE-facilitated antigen presentation.² The persistent engagement of FcεRI/CD23 by IgE, on the other hand, recruits a diverse group of cells (monocytes/macrophages, smooth muscle cells, and B cells) that play important roles in maintaining tissue inflammation.¹

The synthesis of IgE and antibody isotypes other than IgM requires class switching. This process consists of an intrachromosomal DNA recombination that involves

Abbreviations used

ALEX: Allergy and Endotoxin [study]

C_H: Constant heavy chain

repetitive switch regions located upstream of each constant heavy chain (C_H) gene, except C δ , and results in the juxtaposition of different downstream C_H genes to the expressed VDJ transcription unit.³ Switch recombination allows B cells to express Igs with identical antigen specificity but different effector functions. The functional diversification of the antibody response achieved through class switching is essential for a normal immune response, as eloquently shown by the severe immunodeficiency observed in patients with hyper-IgM syndrome, whose B cells can produce antigen-specific IgM but fail to switch to other isotypes.³

The choice of the isotype expressed as a result of class-switch recombination is directed by T_H cell–derived cytokines in combination with antigen-dependent, CD40-mediated B-cell activation.³ The cytokines IL-4 and IL-13, expressed by allergen-activated T_H2 cells, have a unique capacity to induce IgE switching, such that IgE expression provides a molecular signature of T_H2-dependent activation events in both human subjects and mice.¹ Interestingly, *in vitro* studies have shown that human peripheral blood naive B cells stimulated through the IL-4 receptor and CD40 express not only IgE but also IgG1 and IgG4.⁴ IgG3 is also expressed by IL-4-treated, tissue-derived B cells.⁵ Analysis of recombined IgM/IgE genomic switch regions and switch circles excised on class-switch recombination, their complementary products, has revealed that IgE isotype switching in human B lymphocytes occurs both directly, from IgM to IgE, and sequentially, typically through IgG1, IgG4, or both (see Fig E1 in the Online Repository at www.jacionline.org).^{6–8} Notably, because class switching entails looping out and deletion of the DNA located between the switch regions involved in recombination, this process is both irreversible and unidirectional; that is, when occurring sequentially, it can only proceed forward along the locus according to the order in which individual C_H genes are arrayed (C γ 3, C γ 1, C α 1, C γ 2, C γ 4, C ϵ , and C α 2 for the human immunoglobulin locus on chromosome 14).¹

Several epidemiologic studies have shown that environmental influences can potentially affect IgE expression and allergic phenotypes. A wealth of information about the interface between immune responses and the environment has been provided by the Allergy and Endotoxin (ALEX) study, which analyzed a population from rural areas of Germany, Austria, and Switzerland thoroughly defined for lifestyle, environmental exposures, and allergic phenotypes.⁹ Studies in the ALEX populations have clearly shown that exposure to bacterial products abundant in a farming environment, such as endotoxin, is associated with a strong protective effect against atopy and IgE-dependent disease.¹⁰ The protective effects of high bacterial loads on T_H2-dependent disease are a strong argument in

support of the hygiene hypothesis and are thought to reflect the ability of microbial products to activate immunoregulatory responses through the innate immune interface.¹¹

Inasmuch as IgE synthesis by human B cells typically results from allergen-dependent, T_H2-initiated class-switch recombination, the effect of environmental exposures on IgE expression is likely to involve a modulation of isotype switching. Understanding the mechanisms responsible for the protective effect of a farming environment on IgE responses could therefore provide insights into the regulation of isotype switching *in vivo* and propose a paradigm leading to effective preventive and therapeutic strategies.

We assessed the coexpression of allergen-specific serum IgE and IgG subclasses in the ALEX population to reconstruct patterns of allergen-induced isotype switching and determine at which steps in the switching process farm exposure exerts its protective effects. We also asked whether specific isotype switching pathways leading to IgE expression correlate with the prevalence of atopic disease. Our results define the patterns followed by natural allergen-induced antibody responses and their relationship with allergic disease and show that the protective effect of farm exposure was confined to T_H2-dependent immunoglobulin isotypes and were allergen and switch stage specific.

METHODS

Study design

Children included in this study were participants in the cross-sectional ALEX study conducted in rural areas of Germany, Austria, and Switzerland.⁹ All children living on a farm in a predefined area and a random sample of children living in the same area but not raised on a farm were included in the study ($n = 812$). The average age of the participants was 9.5 ± 1.6 years (mean $\pm$ SD), and 51.5% were boys. Approval to conduct the survey was obtained from the 3 local ethics committees for human studies and from the principals of the schools attended by the children. Written informed consent was obtained from the parents of all children.¹⁰

Early and current farm exposure

In the present study currently exposed children were identified as those whose parents answered “yes” to the question “Does your child live on a farm?” Centrally trained fieldworkers interviewed parents to determine the child’s exposure to stables and farm milk in the first year of life. Throughout this article, we included in the farm-exposed group children currently living on a farm at age 9 years, as well as children who were not currently living on a farm but were exposed to stables, farm milk, or both in the first year of life. Children not currently living on a farm and not exposed to stables or farm milk in the first year of life were in the nonfarm group. Characteristics of farming and nonfarming households have been previously described.¹⁰

Allergic disease

The prevalence of physician-diagnosed asthma and hay fever, as well as the occurrence of hay fever and wheezing symptoms during the past 12 months, in the children was assessed by parent-completed questionnaires for the child using standard International Study of Asthma and Allergies in Childhood questions.

Serum IgE and IgG subclasses

Serum levels of IgG1, IgG2, IgG3, IgG4, and IgE to purified recombinant allergens from the most common allergen sources (grass

pollen, house dust mites, and cat) associated with respiratory allergies were measured by means of ELISA, as previously described.¹²⁻¹⁷ Two immunologically unrelated major timothy grass pollen allergens (Phl p 1 and Phl p 5), the major cat allergen (Fel d 1), and the major house dust mite allergen (Der p 2) were chosen as marker allergens for each allergen source. Purified recombinant allergens equivalent to the natural allergens in their IgE reactivity were used in the ELISA experiments.¹⁸⁻²¹ Sera were diluted 1:10 for detection of allergen-specific IgE and 1:300 for detection of specific IgG1, IgG2, IgG3, and IgG4. Murine mAbs with specificity for IgE (clone G7-26, IgG2a, reacting with the human IgE constant region but not other immunoglobulin heavy chains; BD PharMingen, San Diego, Calif), IgG1 (clone JDC-1, IgG1, reacting with human IgG1 but not other immunoglobulin isotypes; BD PharMingen), IgG2 (clone G18-21, IgG1, reacting selectively with human IgG2; BD PharMingen), IgG3 (clone HP-6050, IgG1, reacting selectively with human IgG3; Sigma, St Louis, Mo), and IgG4 (clone JDC-14, IgG1, reacting selectively with human IgG4; BD PharMingen) were used as recommended by the manufacturer to detect allergen-specific human immunoglobulins. Murine mAbs were traced with a 1:1000 dilution of a peroxidase-linked sheep anti-mouse IgG antibody (Amersham Biosciences, Little Chalfont, Buckinghamshire, United Kingdom) that had been affinity adsorbed (to remove cross-reacting antibodies toward rat, human, and rabbit immunoglobulin) and further purified on an affinity mouse IgG column. In addition to the test sera, each ELISA plate included a negative control (serum from a nonatopic individual), a buffer control, and reference sera from 3 allergic patients containing defined levels of allergen-specific IgE, as measured by using quantitative CAP determinations (positive control; Phadia, Uppsala, Sweden). Results for each allergen and immunoglobulin isotype were normalized by including reference sera on each plate to allow a comparison of OD values from different ELISA plates and avoid any potential plate-to-plate variability in the OD measurements. The first of several plates measured for each allergen and isotype in the ELISA reader served as a reference. The OD values for the other plates were normalized by the ratio between the OD of the reference serum from the reference plate (mean of duplicate determinations) and the OD of the reference serum from each of the subsequent plates (mean of duplicate determinations). The cutoff level for a positive IgE measurement was set at 2-fold the OD value obtained for a negative control serum. For IgG subclass measurements, in which buffer and negative sera yielded almost identical OD results, the cutoff level for a positive reaction was set at 2-fold the OD value of the buffer control.

The numbers of available samples varied by allergen and isotype and are provided in Table I. The proportion of children identified as currently living on a farm represented in these samples was consistent, ranging from 36% to 42%. The concentration of total serum IgE was measured in a central laboratory (University Children's Hospital Berlin Charité) by using commercially available kits (CAP; Pharmacia, Uppsala, Sweden).

Statistics

Allergen-specific IgG and IgE data were used as dichotomous variables: the child had either negative or positive results for a given allergen-specific isotype. Total serum IgE values were log-normally distributed, and group differences were assessed by using ANOVA. Contingency tables and Pearson χ^2 statistics were used to compare proportions. When cell frequencies were between 1 and 5, the Fisher exact test was used; statistics were not calculated for contingency tables containing any cells with a frequency of 0. Logistic regression was used to adjust for potential confounders in relation to disease outcomes. *P* values of less than .05 were considered significant. Statistical analyses were performed by using the Statistical

TABLE I. Prevalence of allergen-specific IgG subclasses and IgE at age 9 years by farm exposure

Immuno- globulin isotype	Allergen specificity*	n	No farm exposure	n	Farm exposure†	Pearson χ^2 <i>P</i> value
			% immuno- globulin positive		% immuno- globulin positive	
IgG1	rPhl p 1	233	87.6	371	81.7	.06
	rPhl p 5	233	82.0	371	76.0	.08
	rFel d 1	214	71.0	342	57.0	.001
	rDer p 2	233	83.3	371	78.2	.13
IgG2	rPhl p 1	194	51.5	333	55.0	.4
	rPhl p 5	216	44.4	359	46.2	.7
	rFel d 1	131	65.6	252	63.5	.7
	rDer p 2	194	55.2	333	53.2	.7
IgG3	rPhl p 1	239	11.3	384	13.3	.5
	rPhl p 5	257	8.6	414	6.0	.2
	rFel d 1	257	1.9	414	1.0	.3
	rDer p 2	257	4.7	414	3.6	.5
IgG4	rPhl p 1	261	18.4	487	9.7	.001
	rPhl p 5	261	23.8	487	15.4	.005
	rFel d 1	263	29.3	485	19.0	.001
	rDer p 2	214	16.4	439	13.9	.4
IgE	rPhl p 1	282	23.4	514	8.8	<.001
	rPhl p 5	282	22.3	514	8.2	<.001
	rFel d 1	282	9.6	514	4.9	.01
	rDer p 2	282	2.8	514	6.2	.04

*rPhl p 1 and rPhl p 5 are distinct major timothy grass allergens, rDer p 2 is the major house dust mite allergen, and rFel d 1 is the major cat allergen.

†Farm exposure was defined as currently living on a farm at age 9 years, exposure to stables/farm milk during the first year of life, or both.

Package for the Social Sciences for Windows 14.0 (SPSS, Inc, Chicago, Ill) and STATA 9.0 (StataCorp, College Station, Tex).

RESULTS

A farming environment modifies the expression of IgG1, IgG4, and IgE but not IgG2 and IgG3

In an effort to elucidate the biologic effects of environmental exposure on humoral immunity to allergens, we first examined the effects of farm exposure (defined as currently living on a farm at age 9 years, exposure to stables/farm milk during the first year of life, or both) on allergen-specific IgG subclasses and IgE responses. We chose to assess the response directed against major inhalant allergens from grass, cat, and mite that are implicated in allergic asthma and common in the environment in which the ALEX children live⁹ but are biochemically and immunologically distinct and representative of different modalities of sensitization and exposure. Instead of allergen extracts, which represent mixtures of allergenic and nonallergenic components, we chose to use purified allergenic proteins expressed in recombinant form so as to compare antibody

responses of defined specificity.²² Because we were interested in the effect of farm exposure on the prevalence of allergen-specific antibody responses, IgE and IgG subclass measurements were reported as a dichotomous variable (detectable vs undetectable).

Table I shows that, consistent with previous reports, the prevalence of IgE responses against the 2 grass allergens (rPhl p 1 and rPhl p 5) at age 9 years was substantial among nonfarm children (23.4% and 22.3%, respectively) but significantly lower among farm-exposed children (8.8% and 8.2%, respectively; $P < .001$). IgE antibodies to cat (rFel d 1) were found overall less frequently, and again, the prevalence of IgE responses was significantly lower in the farm-exposed group (4.9%) compared with in the unexposed group (9.6%, $P = .01$). IgE responses to dust mite (rDer p 2) were also relatively rare, but surprisingly, farm exposure was associated with a modest but significant increase in their prevalence (6.2%) compared with that seen in the nonfarm group (2.8%, $P = .04$). The prevalence of allergen-specific IgG responses varied for different subclasses, with IgG1 having the highest and IgG3 having the lowest prevalence. Interestingly, a strong protective effect of farm exposure was observed for IgG1 to rFel d 1 and IgG4 to rPhl p 1, rPhl p 5, and rFel d 1 but not rDer p 2. In contrast, IgG2 and IgG3 responses were unaffected by farm exposure, regardless of the allergen examined. These data show that inhalant allergens induce a wide spectrum of antibody isotypes. However, the effects of exposure to a farm environment appear to be allergen specific and restricted to the T_H2-dependent isotypes IgG1, IgG4, and IgE.

Effects of farming exposure on patterns of allergen-induced isotype switching *in vivo*

To reconstruct the steps followed by allergen-induced, T_H2-dependent isotype switching and define the levels at which a farming environment acts to protect against IgE responses, we next assessed coexpression of allergen-specific IgG1, IgG4, and IgE at age 9 years and overlaid the effect of farm exposure on the model thus generated.

Fig 1, A, shows that for virtually all the ALEX children, switching to IgE in response to the grass allergen rPhl p 1 was marked by the expression of IgG1. A subset of IgG1-positive children also expressed IgG4 (15.9%), and an even smaller subset expressed IgG4 and IgE (6.2%). Of note, the majority of IgE responses to rPhl p 1 occurred in the absence of IgG4 (66.7%). Interestingly, only a few children failed to express rPhl p 1-specific IgG1 (14.2%), and nearly all of these children also failed to express IgG4 and IgE, suggesting switching to IgE or IgG4 in response to this antigen rarely occurs in the absence of IgG1.

Fig 1, A, also shows that farm exposure targeted multiple steps in the rPhl p 1-specific switching pathway. A farming environment was associated with a significant decrease in the prevalence of IgG1 responses and in the expression of IgG4 in IgG1-positive children. Moreover, strong protection was observed against expression of IgE in both IgG4-positive and IgG4-negative children. A

similar analysis of rPhl p 5-specific antibody responses yielded comparable results (see Fig E2 in the Online Repository at www.jacionline.org).

Similar to grass allergen, only a subset of IgG1-positive children also expressed IgG4 (36.0%) to rFel d 1, and a smaller subset also expressed IgG4 and IgE (7.8%). However, the majority of IgE responses to rFel d 1 occurred in the presence of IgG4 (79.4%). Surprisingly, farm exposure had a different effect on isotype-switching patterns in response to rFel d 1. Fig 1, B, shows that farming provided strong protection against IgG1 responses but only modest protection against expression of IgE in IgG4-positive children. Most importantly, the prevalence of IgG4 responses in IgG1-positive subjects, an important target of farm exposure in humoral immunity to grass, was unaffected by farming. Similar to rPhl p 1, no significant IgG4 responses, IgE responses, or both were detected in the absence of IgG1, but the proportion of IgG1-negative children was substantially higher for rFel d 1.

Yet another pattern was observed for rDer p 2 (Fig 1, C). Indeed, antibody responses to this allergen appeared to occur independently of farm exposure, with the exception of a modest protective effect on IgG1 responses. Similar to rPhl p 1, rPhl p 5, and rFel d 1, IgE and IgG4 responses were nearly absent in IgG1-negative children.

These results show that IgE responses to natural inhalants typically co-occur with IgG1 and more rarely also IgG4. Furthermore, our data suggest that exposure to a farming environment modulates allergen-dependent isotype switching by acting at distinct levels in pathways initiated by distinct allergens.

Association between IgE-positive allergen-specific isotype patterns and allergic disease

Individuals who produce allergen-specific IgE are known to be at an increased risk of allergic disease.²³ Nearly all the ALEX children who expressed allergen-specific IgE also expressed allergen-specific IgG1 (Fig 1), but only a subset of the children who expressed IgG1 and IgE also produced IgG4. We asked whether the prevalence of allergic disease differed between the children who produced allergen-specific IgG1, IgG4, and IgE and those who produced only IgG1 and IgE. Table II shows the prevalence and adjusted odds ratios of diagnosed asthma and hay fever, as well as current symptoms during the previous 12 months for the 2 groups. Children who produced rPhl p 1-specific IgG1, IgG4, and IgE had significantly more diagnosed asthma and hay fever, as well as symptoms during the previous year, compared with children who produced IgG1 and IgE alone. After adjusting for current farm living and exposure to stables/farm milk in the first year of life, the odds ratio for wheezing symptoms during the previous 12 months was 3.9 (95% CI, 1.3-11; $P = .01$), and the odds ratio for hay fever symptoms during the previous 12 months was 4.9 (95% CI, 1.8-14; $P = .002$) for children who produced IgG4 in addition to IgG1 and IgE compared with those who did not. A similar pattern was observed for rPhl p 5 and disease. These results show that coexpression of grass-

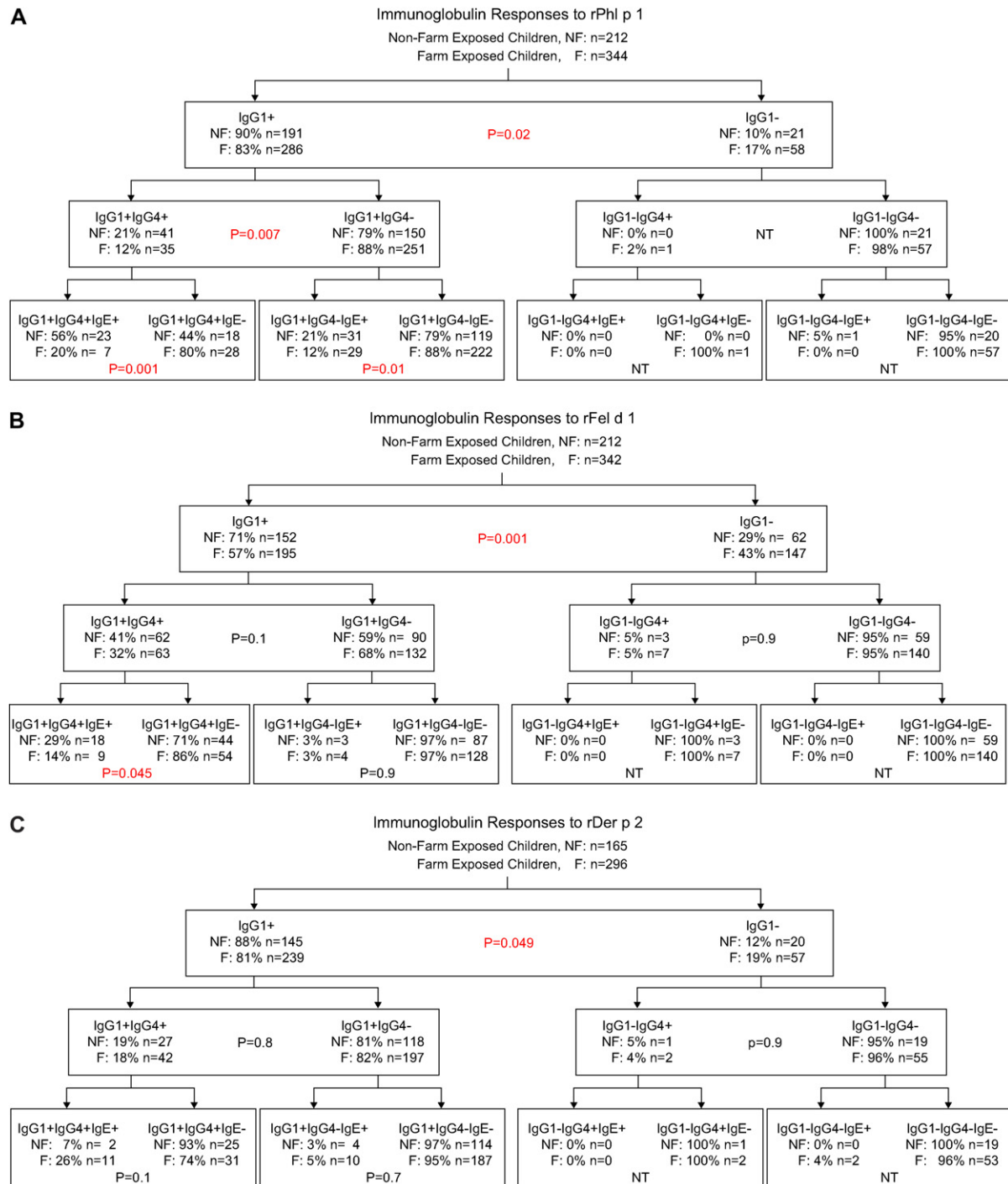


FIG 1. Effects of farm exposure on patterns of immunoglobulin production to rPhl p 1 (A), rFel d 1 (B), and rDer p 2 (C). F, Farm exposure; NF, no farm exposure. The P values are associated with the comparison of response rates between farm-exposed and non-farm-exposed children by means of χ^2 analysis. Significant P values are reported in red. For comparisons with response rates of 0, no statistics were computed (NT).

specific IgG1, IgG4, and IgE is associated with a significantly increased risk of allergic disease compared with production of IgG1 and IgE without IgG4, independent of farm exposure.

The relationships between allergen-specific responses to rFel d 1 and rDer p 2 and allergic disease are presented in Table II. Interestingly, coexpression of IgG4 and IgE to mite appeared to be associated with increased disease,

TABLE II. Prevalence and risk of allergic disease associated with IgE isotype groups

Allergen-specific isotype groups*	Hay fever diagnosis			Runny nose and itchy eyes in past 12 mo			Asthma diagnosis			At least 1 wheeze attack in past 12 mo		
	N	Percent†	OR‡ (95% CI)	N	Percent†	OR‡ (95% CI)	N	Percent†	OR‡ (95% CI)	N	Percent†	OR‡ (95% CI)
rPhl p 1												
IgG1 ⁺ IgG4 ⁻ IgE ⁺	59	25.4	1.0	59	30.5	1.0	60	11.7	1.0	60	13.3	1.0
IgG1 ⁺ IgG4 ⁺ IgE ⁺	30	63.3	4.0 (1.5-11)	29	72.4	4.9 (1.8-14)	30	33.3	3.2 (1.0-9.9)	29	41.4	3.9 (1.3-11)
P value		<.001	.006		<.001	.002		.01	.045		.003	.01
rPhl p 5												
IgG1 ⁺ IgG4 ⁻ IgE ⁺	33	15.2	1.0	33	24.2	1.0	33	9.1	1.0	33	12.1	1.0
IgG1 ⁺ IgG4 ⁺ IgE ⁺	47	59.6	8.5 (2.6-28)	47	61.7	5.2 (1.8-15)	48	29.2	5.1 (1.2-21)	47	27.7	3.3 (0.9-12)
P value		<.001	<.001		.001	.002		.03	.03		.09	.07
rFel d 1												
IgG1 ⁺ IgG4 ⁻ IgE ⁺	7	28.6	1.0	7	42.9	1.0	7	57.1	1.0	7	42.9	1.0
IgG1 ⁺ IgG4 ⁺ IgE ⁺	26	30.8	0.4 (0.03-4.7)	24	37.5	0.5 (0.06-3.3)	27	22.2	0.2 (0.03-1.3)	26	29.9	0.5 (0.07-2.9)
P value		.9	.4		.9	.4		.2	.08		.6	.4
rDer p 2												
IgG1 ⁺ IgG4 ⁻ IgE ⁺	14	7.1	1.0	14	28.6	1.0	14	7.1	1.0	14	28.6	1.0
IgG1 ⁺ IgG4 ⁺ IgE ⁺	12	16.7	2.6 (0.2-34)	13	53.8	3.4 (0.6-18)	13	30.8	5.6 (0.5-59)	12	41.7	1.7 (0.3-8.9)
P value		.6	.5		.3	.2		.2	.2		.7	.5

OR, Odds ratio.

*rPhl p 1 and rPhl p 5 are distinct timothy grass allergens, rDer p 2 is a house dust mite allergen, and rFel d 1 is a cat allergen.

†Unadjusted percentage of IgE-positive group with disease; P value from Pearson χ^2 (grass) or Fisher exact test (cat and mite) statistics for comparison of percentages of each IgE-positive group with disease.

‡Odds ratios for disease adjusted for current farming status and any exposure to stables/farm milk in the first year of life; P value for the adjusted odds ratio.

similar to anti-grass responses. In contrast, similar to previous reports,²⁴ the data suggested a protective effect against allergic disease of IgG4/IgE coexpression in response to cat. However, because of the small IgE-positive group sizes, the power for these analyses is low, and the results should not be considered conclusive.

Association between IgE-positive, allergen-specific isotype patterns and total serum IgE levels

Because not only the presence of allergen-specific IgE but also the amount of IgE produced is associated with the risk of disease,²⁵ we then asked whether there was a quantitative difference in IgE responses depending on the pattern of allergen-specific IgE production. More specifically, we asked whether the children who coexpressed IgG1, IgE, and IgG4 had higher levels of total serum IgE compared with levels in children who coexpressed only IgG1 and IgE, a feature that could help explain the relation we observed between isotype production patterns and disease. Children who produced IgG1, IgG4, and IgE to rPhl p 1 had significantly higher levels of total serum IgE compared with those seen in children who produced IgG1 and IgE without IgG4 (Table III). A similar relation was observed for rPhl p 5. These data show more intense IgE responses in individuals who coproduce IgG4 and IgE, indicating that IgE and IgG4 responses to natural allergens are not independently regulated. Increased prevalence of IgE-mediated disease in children coexpressing IgG1, IgG4, and IgE might therefore result from more

TABLE III. Total serum IgE levels associated with IgE isotype groups

Allergen-specific isotype groups*	N	Total serum IgE level (IU/mL)	
		GM† (95% CI)	P value‡
rPhl p 1			
IgG1 ⁺ IgG4 ⁻ IgE ⁺	61	128 (98-190)	<.001
IgG1 ⁺ IgG4 ⁺ IgE ⁺	30	473 (295-757)	
rPhl p 5			
IgG1 ⁺ IgG4 ⁻ IgE ⁺	33	114 (69-189)	.003
IgG1 ⁺ IgG4 ⁺ IgE ⁺	48	341 (212-548)	
rFel d 1			
IgG1 ⁺ IgG4 ⁻ IgE ⁺	8	245 (95-634)	.5
IgG1 ⁺ IgG4 ⁺ IgE ⁺	27	165 (96-284)	
rDer p 2			
IgG1 ⁺ IgG4 ⁻ IgE ⁺	14	225 (131-387)	.03
IgG1 ⁺ IgG4 ⁺ IgE ⁺	13	480 (294-786)	

*rPhl p 1 and rPhl p 5 are timothy grass allergens, rDer p 2 is a house dust mite allergen, and rFel d 1 is a cat allergen.

†Geometric mean and 95% CI for the mean.

‡One-way ANOVA.

active IgE production and IgE-mediated inflammation. Coexpression of IgG4 and IgE to rDer p 2 appeared to be associated with higher total IgE levels, similar to grass-specific responses, whereas total serum IgE levels appeared to be lower in children expressing anti-cat

IgG4 and IgE. Again, the results should be interpreted with caution because of the small group sizes.

DISCUSSION

Basic biology and epidemiology are distinct but complementary approaches to understanding disease pathogenesis. The biologic significance of mechanistic studies in model systems ultimately depends on their ability to stand the test of validation in large human populations. Epidemiologic analysis, on the other hand, needs to be constrained so as to integrate the inner workings of biologic systems. Over the past decade, cellular and molecular studies have explored the signals and mechanisms that regulate human isotype switching to IgE, and epidemiologic studies have highlighted the complex effects of environmental exposures on the allergic response. We reasoned that the mechanistic studies of class-switch recombination could provide a robust framework to interpret the epidemiology of allergen-specific antibody responses, whereas epidemiologic analysis could highlight fundamental regulatory facets of the switching process as it occurs *in vivo*. By framing epidemiologic analysis within state-of-the-art molecular paradigms, we analyzed patterns of allergen-induced switching in a large population well characterized in terms of exposures, lifestyle, and IgE-dependent disease. The use of recombinant allergens allowed us to identify responses of defined and comparable specificity. The results reported herein reveal novel features of allergen-induced antibody responses, their modulation by exposure, and their relationship with disease.

The first surprise came from the observation that for all the allergens examined, virtually no IgE was detected without a concomitant IgG1 (and, less frequently, IgG4) response of the same specificity. Furthermore, virtually no one produced allergen-specific IgG4 without IgG1. Although only 1 serum sample was available for each ALEX study child and thus our results provide a cross-sectional rather than a longitudinal view of allergen-specific antibody responses, these findings suggest that expression of IgG1 might be a prerequisite for switching to C_H genes, such as IgG4, IgE, or both, positioned more downstream in the Ig locus. More generally, the coexistence of IgG1, IgG4, and IgE responses of the same allergen specificity, combined with a prevalence pattern that closely reflects the location of the corresponding C_H genes in the Ig locus (IgG1 more prevalent than IgG4, IgG4 more prevalent than IgE), suggests that ongoing *in vivo* expression of T_H2-dependent Ig isotypes in response to recurrent or continuous allergen exposure might be regulated coordinately and sequentially. The finding that farm exposure exerts its effects at discrete stages of T_H2-dependent IgG1, IgG4, and IgE switching indicates that distinct signals regulate the transition from one isotype to the other *in vivo*. On the other hand, the inability of exposure to affect IgG2 and IgG3 responses implies that the latter are likely controlled by different mechanisms that are thus far

unknown. These novel aspects of allergen-induced humoral immunity would not have emerged had we limited our analysis to the global relationship between farm exposure and IgE responses rather than dissect the effect of the environment on distinct steps of isotype switching.

As importantly, at variance with the common notion that allergens are immunogenic only for selected and genetically predisposed individuals, only a minority of ALEX children failed to express IgG1 to grass and mite, and the same was true for anti-cat IgG1, although the nonresponsive group was larger. These results suggest that interindividual (eg, genetically determined) differences in allergen responsiveness are more likely to affect the profile of antibody responses than the ability to initiate a response.

Analysis of the interaction between isotype switching pathways and exposure to a farming environment revealed unexpected complexities of the allergen-specific response. Indeed, each allergen-induced pathway was influenced by exposure according to a distinct pattern.

Farm exposure protected against grass-specific responses at every step along the IgG1/IgG4/IgE switching pathway, such that the strong global protection against IgE responses detected in the farming population appeared to result from multiple and possibly synergistic effects at distinct steps within the process. In contrast, protection from responses to cat was concentrated mostly at the IgG1 level, whereas farm exposure had no significant effect on antibody responses to mite. Because patterns of isotype switching to different allergens were assessed within the same population, these findings are unlikely to result from interindividual differences in genetic background, allergen exposure conditions, or both. Furthermore, although the contexts of exposure (outdoor vs indoor) were different, all of the test allergens were inhaled, making it unlikely that tissue district-specific differences in dendritic cell function could account for the different patterns of isotype switching we observed. Rather, the allergen-specific patterns of antibody responses detected in our study are likely to result from profound and thus far unappreciated peculiarities in the biology of allergen/immune system interactions. For instance, it is possible that cat dander, mite feces, and pollen release nonallergenic components that might influence the resulting immune response.

Our work also revealed a complex relationship between isotype switching patterns and prevalence of IgE-mediated disease. Indeed, the risk for allergic disease was substantially increased in individuals coexpressing grass-specific IgG1, IgG4, and IgE compared with those who did not express IgG4. The relationship between the presence of grass-specific IgG4 and the increased prevalence of disease in the ALEX population appeared to be mediated by the expression of markedly higher levels of IgE, such that IgG4 expression in IgE-positive individuals might have signaled a more robust activation of allergen-dependent T_H2-mediated events. This enhanced activation, rather than the presence of allergen-specific IgG4 per se, likely contributed to higher disease prevalence in children expressing both IgG4 and IgE.

Recent studies in the ALEX population have provided strong support for the notion that allergic diseases represent the outcome of strong environmental and genetic influences.^{26,27} Therefore preventive and therapeutic manipulations of environmental conditions are desirable and might be effective but will require a profound understanding of the pathways through which the environment affects disease mechanisms within a given genetic context. Our epidemiologic tools have identified novel features of the interaction between allergens and the humoral response and novel levels at which farm exposure exerts its protective effects. Although further analyses in a larger population are necessary to decipher the effect of genetic determinants, the unique interactions between farm exposure and antibody responses to specific allergens detected in the ALEX population suggest that interventions aimed at arresting the march of allergen-induced class-switch recombination toward the IgE isotype might need to be tailored to specific target antigens. Our work therefore outlines a tractable paradigm for future mechanistic and clinical studies.

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