

Immunoglobulin G4-antibodies to rBet v 1 and risk of sensitization and atopic disease in the child

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Summary

Background In 1993 extremely high levels of birch-pollen were recorded in Stockholm, Sweden. This provided an opportunity to evaluate the effects of aeroallergen exposure (exp.) on the early immune response.

Objective To assess the influence of exp. to birch-pollen during pregnancy and infancy on the allergen-specific IgE- and IgG4-antibody (ab) response and the development of atopic disease in children.

Methods A total of 970 children with atopic heredity and born in Stockholm 1992, 1993 or 1994 were investigated at age 4.5–5 years. They were divided into five groups; high-dose exp. at 1 year of age, high-dose exp. at 0–3 months, low-dose exp. at 0–3 months, high-dose exp. during pregnancy and low-dose exp. during pregnancy. The children were examined and skin prick tested with inhalant and food allergens. IgE abs (against birch-pollen and recombinant Bet v 1 (rBet v1)) and IgG4 abs (against rBet v 1) were analysed in serum. All children were assembled in one group to assess the effects of different ab responses (IgE/IgG4) on the development of atopic disease.

Results Children exposed to high doses of birch-pollen during the first 3 months of life more often had detectable levels of IgG4 abs to rBet v 1 than the children in the other groups ($P < 0.001$), independent of sensitization to birch. Overall, the risk of allergic rhinoconjunctivitis was increased among children sensitized to birch-pollen and appeared more pronounced in children without detectable levels of IgG4 ab to rBet v 1 (Odds ratio 9.4; 95% Confidence interval: 5.5–16.1). IgE sensitization to birch-pollen seemed to have a stronger influence on the development of atopic disease than the IgG4-ab response.

Conclusion Exposure to high doses of inhalant allergens during the early postnatal period is associated with detectable levels of allergen-specific IgG4 ab even at 5 years of age. An immune modulating effect by IgG4 on symptoms of allergic rhinoconjunctivitis is suggested in children sensitized to birch.

Keywords aeroallergens, atopy, birch, children, IgG subclass antibodies, sensitization

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Introduction

Over the past decades, the prevalence of allergic diseases in childhood has increased [1, 2]. The aetiology is complex, involving genetic predisposition [3] and also environmental factors such as allergen exposure (exp.), maternal smoking, early infections, breastfeeding, birth order and other lifestyle factors [4–9]. The type and dose of allergen and the timing of exp. early in life might be important [10–12]. Moreover, atopic disease of the mother may have an influence on the child's immune response [13–16], and it has been suggested that trans-placental transfer of allergens and immune factors may occur during pregnancy [17, 18].

The role of IgG-antibodies (abs) has also been discussed based on results obtained in both animal models [19] and clinical studies [20–23]. It has been suggested that both IgE and IgG4 abs are markers of sensitization and atopic disease [20, 22], while others have argued that IgG4 abs are primarily involved in the development of tolerance [24].

In the spring of 1993, birch-pollen levels in Stockholm were more than 50 times higher than in 1994. We have previously reported that exp. to high levels of birch-pollen in early infancy (0–3 months) increases the risk of sensitization to the same allergen and symptoms of allergic asthma at 4.5–5 years, but the effects of exp. to high levels of birch-pollen during pregnancy appear milder [10, 25].

In this study we wanted to evaluate the effect of exp. to high doses of birch-pollen during pregnancy or infancy on the IgG4-ab response and the IgE sensitization at 5 years of age. Secondly, we tried to relate the different immune responses

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(IgG/IgE) to development of atopic disease for the whole group of children.

Methods

Study population

Children born in Stockholm County and living in south-western Stockholm (Brännkyrka, Skärholmen, Hägersten, Huddinge and Botkyrka municipalities) were investigated. Five groups of children, 3964 in all, were identified from birth records: children born between February and April of 1992 (children exposed at 1 year of age), children born the corresponding periods of 1993 or 1994 (children exposed during first 3 months of life) and children born between July and October of 1993 or 1994 (mothers exposed during pregnancy) (Table 1). When the children were 4 years old, the families were approached with a questionnaire concerning allergic symptoms in the children and their parents. Answers were obtained for 3288 children (83.0%), and 1826 of these reported a family history (one or both parents) of atopic disease including asthma, allergic rhinoconjunctivitis, atopic dermatitis and/or food allergy. Among these, 1265 children were randomly selected and invited to participate in the study. From this group a total of 295 declined to participate or did not come to the clinical examination, which left 970 children for clinical assessment. They were divided into five groups: high-dose exp. at 1 year of age, high-dose exp. at 0–3 months, low-dose exp. at 0–3 months, high-dose exp. during pregnancy and low-dose exp. during pregnancy. The study

population is described in greater detail in our previous publications [10, 16, 25].

All children in the study (the five birch-pollen exp. groups) were assembled into one group to address the effects of the four different combinations of immune susceptibility (IgG4/IgE) on atopic symptoms.

The local ethics committee at Huddinge University Hospital approved the study. Informed consent was obtained from parents and children.

Medical examination

All children were examined at the age of 4.5–5 years in the paediatric allergy clinic at Huddinge University Hospital, by the same paediatrician (A. K.). The parents were asked to complete a questionnaire about atopic symptoms in the child as well as social and environmental factors, i.e., early infections, number of siblings, pets in the household, duration of breastfeeding and parental smoking. Each child was clinically classified with respect to asthma, allergic rhinoconjunctivitis. Bronchial asthma was defined as three or more episodes of wheezing, or any episode of wheezing if related to exp. to pollen and/or furred pets. Among the children with bronchial asthma, allergic asthma was diagnosed if the children reacted with wheezing when exposed to pollen and/or furred pets, during a period free of airway infection. Allergic rhinoconjunctivitis was diagnosed if rhinitis and/or conjunctivitis appeared at least twice after exp. to a particular allergen and/or seasonal exp. and was unrelated to an infection. Atopic dermatitis was defined according to Hanifin and Rajka [26]. Atopic disease was defined as symptoms of

Table 1. Selection of children and sensitization to birch-pollen in the five exposure groups of the study

	Exposure to birch-pollen				
	High-dose exposure at 1 year Children born in 1992	High-dose exposure at 0–3 months Children born in 1993	Low-dose exposure at 0–3 months Children born in 1994	High-dose exposure during pregnancy Children born in 1993	Low-dose exposure during pregnancy Children born in 1994
Screening stage					
Selected for screening	735	781	721	863	864
Responded to screening questionnaire	619 (85.3%)	666 (85.3%)	598 (82.9%)	666 (77.2%)	739 (86.0%)
Atopic heredity by history	305	367	351	375	428
Clinical assessment					
Invited*	254	242	231	269	269
Declined participation	33	32	13	58	59
Did not come to examination	30	13	23	22	12
Final study group	191	197	195	189	198
Male/female	103/88	92/105	88/107	98/91	102/96
Skin prick test					
Test done	190 (99.5%)	197 (100.0%)	194 (99.5%)	187 (98.9%)	195 (98.5%)
Positive birch response (≥ 3 mm)	27 (14.2%)	35 (17.8%)	17 (8.8%)	14 (7.6%)	9 (4.6%)
RAST birch					
Test done	169 (88.5%)	182 (92.4%)	179 (91.8%)	183 (96.8%)	194 (98.0%)
Positive test	25 (14.8%)	29 (15.9%)	19 (10.6%)	14 (7.6%)	12 (6.2%)
RAST rBet v 1					
Test done	170 (89.0%)	185 (93.9%)	180 (92.3%)	179 (94.7%)	195 (98.5%)
Positive test	25 (14.7%)	29 (15.7%)	19 (10.0%)	13 (7.3%)	11 (5.6%)
Any sensitization to birch	33 (17.4%)	38 (19.3%)	24 (12.4%)	16 (8.7%)	12 (6.2%)

*Randomly selected among those with atopic heredity, who answered a screening questionnaire.

bronchial asthma and/or allergic rhinoconjunctivitis and/or atopic dermatitis in the child.

Skin prick test

Skin prick test (SPT) was performed in the children, against inhalant and food allergens. All SPTs were performed by the same nurse, on the volar side of the forearm according to the manufacturer's instructions (ALK, Copenhagen, Denmark). The SPT included allergens from birch, timothy grass, cat, dog, horse, *Dermatophagoides pteronyssinus*, *Cladosporium* species (Soluprick, 10 histamine equivalent potency, ALK), egg white (Soluprick, weight to volume ratio 1/100), codfish (Soluprick, 1/20), peanut (Soluprick, 1/20), hazelnut (Soluprick, 1/20), cow's milk (3% fat, standard milk) and soy bean protein (Soluprick, 1/20). Histamine chloride 10 mg/mL was used as a positive control, and the allergen diluent as the negative control. A weal diameter ≥ 3 mm recorded after 15 min was considered positive. In accordance with Pepys' definition [27], an individual with at least one positive SPT was classified as atopic.

Immunoglobulin E and immunoglobulin G4 assays

A blood sample was obtained from the arm vein after local anaesthesia (EMLA[®], Astra-Zeneca, Södertälje, Sweden). The blood was centrifuged at 1200g and serum was separated and stored at -18°C . Circulating IgE abs against birch-pollen and rBet v 1 were determined with the Pharmacia CAP-system (Pharmacia Upjohn, Uppsala, Sweden). An IgE-ab level ≥ 0.35 kU/L was considered significant.

For detection of specific anti-Bet v 1 IgG4-ab in human sera with ELISA, Microtitre plates (Costar # 3590 96, Costar Corning, Inc., Corning, NY, USA) were coated with rBet v 1 to an amount of 50 μL /well (1 $\mu\text{g}/\text{mL}$), and incubated at 4°C overnight. The plates were then blocked with 200 μL phosphate-buffered saline (PBS) supplemented with 0.5% bovine serum albumin (BSA) and further incubated for 2 h at 37°C . The plates were then washed once with PBS and 0.05% Tween. Samples (diluted 1/20) and standard were added and incubated at 37°C for 3 h. The plates were then washed four times with PBS and 0.05% Tween. In the following step, mouse monoclonal biotinylated anti-human IgG4 (Sigma clone HP-6025, Sigma-Aldrich Co., St Louis, MO, USA) was added to an amount of 50 μL (diluted 1/2000) and incubated at 4°C overnight, and then washed four times with PBS and 0.05% Tween. Streptavidin-alkaline phosphatase (ALP) (MAB-Tech AB, Stockholm, Sweden) was diluted 1/2000, and 50 μL were added and incubated for 1 h at room temperature. The plates were washed four times with PBS and 0.05% Tween. Finally, the substrate *p*-nitro-phenyl-phosphatase disodium (Sigma, St Louis, MO, USA) was added. The absorbance at 405 nm was measured with an ELISA plate reader (Labsystem Multiscan, Helsinki, Finland). A standard curve was constructed from a serum displaying high amounts of IgG4-specific rBet v 1 antibodies. This standard serum was diluted from 1/200 in two-step dilutions to 1/3200. The highest standard point was given the arbitrary value of 200 U. The value for each serum was calculated from the constructed curve following correction for dilution. The standard and serum samples were analyzed in duplicates. Detection limit for IgG4-ab response was ≥ 1.2 U/L.

Pollen measurements

Pollen counts have been recorded continuously since 1973 at the Swedish Museum of Natural History in Stockholm, using a Burkard trap. In this suction trap, pollen enters a narrow orifice, directed into the wind, and adheres to a Vaseline- and glycerine-coated celluloid tape on a slowly rotating drum. Once daily, the tape is transferred to microscope slides and examined for estimation of the pollen content of the air [28].

Statistical analysis

χ^2 analyses were used to compare children in the five birch-pollen exp. groups. All 970 children in the study were assembled into one group to assess the associations of the immune-susceptibility groups (IgG4/IgE) and atopic manifestations; thus, combined effects, IgG4⁺/IgE⁺, IgG4⁺/IgE⁻ and IgG4⁻/IgE⁺ vs. IgG4⁻/IgE⁻ (the reference group), were evaluated. Odds ratios and 95% confidence intervals were calculated for symptoms of atopy in the children. Data were adjusted for differences in background variables using multiple logistic regression analysis. These variables reflected the child's situation after birth: breastfeeding exclusively during the first 4 months of life, maternal smoking and furred pets (cats, dogs and rodents) in the household. In addition, data were adjusted for gender and birch-pollen exp. groups. Data were analysed by using Stata 8.0 software (Stata Corporation, College Station, TX, USA).

Results

The total pollen counts reported in Stockholm for the three spring seasons (April–May, mainly birch-pollen) were 2194 counts/m³ in 1992, 26 805 counts/m³ in 1993 and 435 counts/m³ in 1994. (Fig. 1).

Demographic data on the five groups of children have been presented in earlier publications (10, 16 and 25). The groups were comparable regarding breastfeeding and exp. to pets. However, the mothers of the children born in 1994 smoked less during pregnancy and the first 3 months of the baby's life, than the mothers of the children born in 1993. The number of older siblings and family history of asthma, atopic eczema or food allergy did not differ between groups. Children high-dose exposed to birch-pollen in early infancy were more often sensitized to birch, and they had increased occurrence of allergic asthma compared with the other groups, as described in earlier publications [10, 16, 25]. The results from skin prick tests, analyses of IgE-abs against birch-pollen and recombinant Bet v1 are presented in Table 1.

Immunoglobulin E and Immunoglobulin G4 sensitization

Children high-dose exposed to birch-pollen in early infancy more often had detectable levels of IgG4 ab to rBet v1 (36.5%) than the other groups of children (11.9–17.8%, $P < 0.001$; Fig. 2). This was observed both for children sensitized to birch (positive SPT to birch and/or RAST for birch/rBet v 1) and non-sensitized children (43.8% and 34.9%, respectively; Fig. 3). There was no strong association

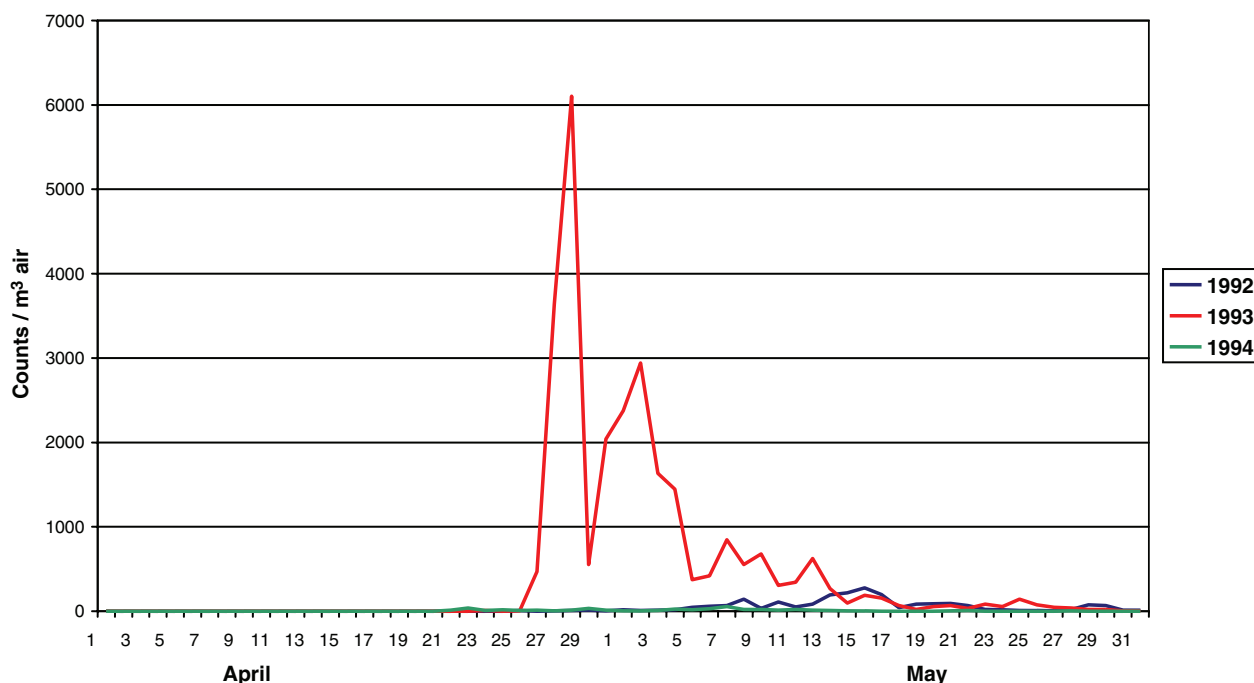


Fig. 1. Total pollen counts for the three spring seasons (April–May, mainly birch-pollen). 2194 counts/m³ in 1992, 1993:26 805 counts/m³, and 1994:435 counts/m³ air.

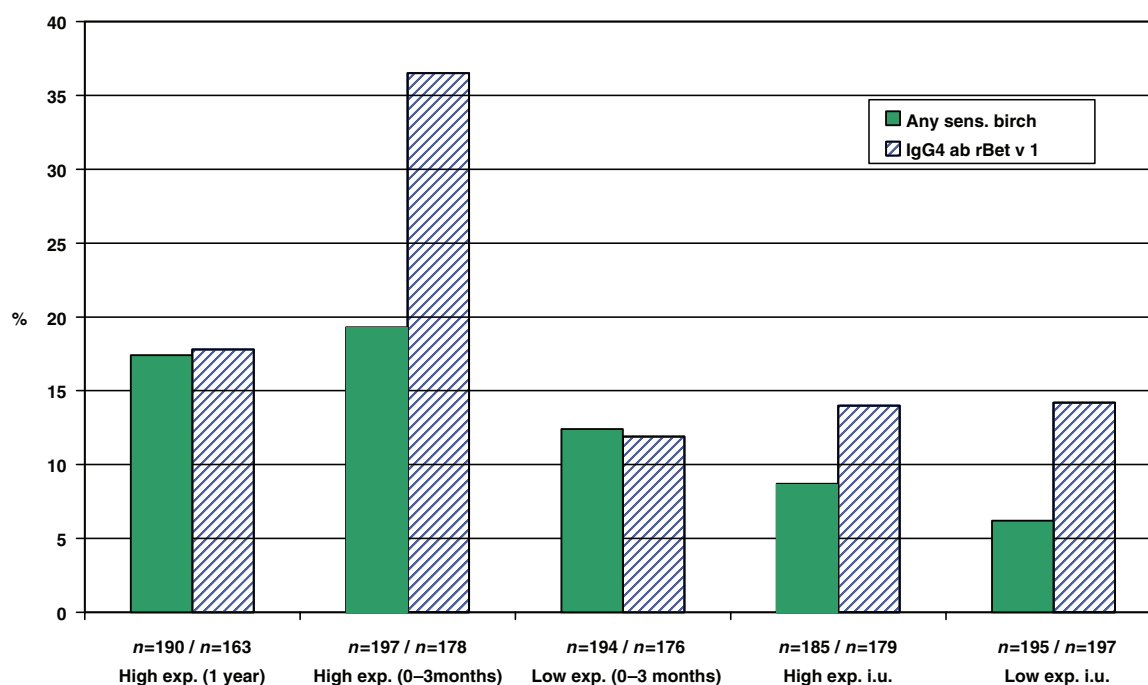


Fig. 2. Number of children sensitized to birch and/or with detectable levels of IgG4, in relation to birch-pollen exposure.

between sensitization to birch and detectable levels of IgG4 to rBet v 1 on an individual level (data not shown). Among the low-dose-exposed children, the birch-sensitized children appeared more often to have detectable levels of IgG4 ab than the non-sensitized children, and this was observed both for those exposed to birch-pollen during pregnancy ($P = 0.05$) and for those exposed in the first 3 months of life ($P < 0.005$; Fig 3). Not all children had detectable levels of IgG4 abs, but

among those who did, the geometric mean values did not differ between the five exp. groups (data not shown).

ImmunoglobulinG4/ImmunoglobulinE and symptoms of atopic disease

All children in the study (the five birch-pollen exp. groups) were assembled in one group to assess the relations between

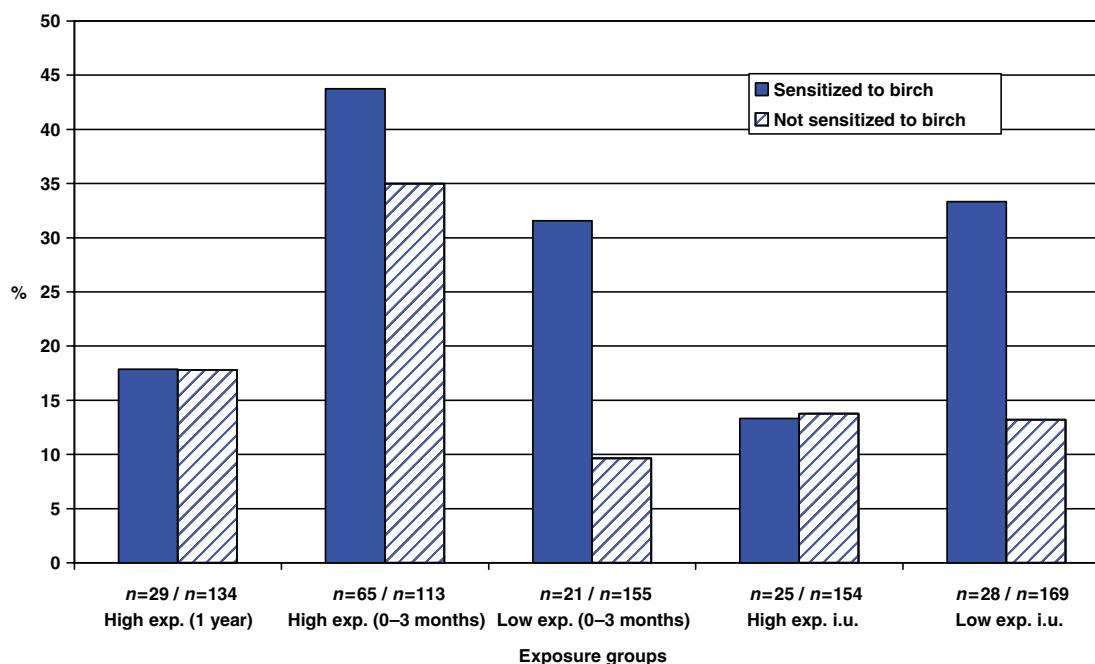


Fig. 3. Positive IgG4 response in birch-sensitized and not birch-sensitized children, in relation to birch-pollen exposure.

IgE sensitization to birch (positive SPT and/or RAST for birch and/or rBet v 1), IgG4 abs to rBet v 1 and symptoms of allergic disease (Table 2). Non-sensitized children without detectable IgG4-ab response (IgG4⁻/IgE⁻) were used as a reference group. The risk of allergic rhinoconjunctivitis was increased among children sensitized to birch-pollen with detectable IgG4-ab response (IgG4⁺/IgE⁺; Odds ratio (OR) 5.5; 95% Confidence interval (CI): 2.5–12.2), and this appeared to be even more pronounced in birch-sensitized children without detectable levels of IgG4 ab to rBet v 1 (IgG4⁻/IgE⁺; OR 9.4; 95% CI: 5.5–16.1; Table 2). This was primarily evident among those with heavy exp. to birch-pollen (not shown in table). Because of small numbers it was not possible to analyse the effects on rhinoconjunctivitis in relation to IgG4/IgE response for the five birch-pollen exp. groups separately. Furthermore, there was no association between increasing IgG4 levels and risk of developing rhinoconjunctivitis in birch-sensitized or not birch-sensitized children (data not shown).

No apparent differences in risk of asthma or atopic dermatitis related to IgG4-ab response were seen in those sensitized to birch.

Discussion

In this study, we investigated the significance of specific IgG4- and IgE-ab response to birch-pollen in relation to exp. during pregnancy and early infancy and the development of atopic disease in children at 5 years of age.

Primarily we looked at IgG4-ab response and IgE sensitization. Our data showed that among 5-year-old children with a family history of atopy, a short high-dose exp. to birch-pollen during the first 3 months of life resulted in an increased

number of individuals with detectable IgG4 abs against rBet v 1. This was in contrast to children exposed to low doses of birch-pollen at the same age, children high- or low-dose exposed during pregnancy or children high-dose exposed at 1 year of age. These observations might indicate that IgG4 could be a marker of early allergen exp. in general, a finding that is in agreement with previous publications on antigen exp. and IgG-antibody (ab) responses [23]. No strong association between sensitization to birch and detectable levels of IgG4 to rBet v 1 could be observed. Children high-dose exposed to birch-pollen during the first 3 months of life had increased IgE sensitization to birch, as previously reported [10], but it was not always the same individuals who were IgE-sensitized to birch and who had detectable levels of IgG4 to rBet v 1. Thus, our results could not confirm previous studies where IgG4 has been suggested to exert an immune-modulating effect attenuating IgE production as proposed by Platts Mills et al. for high-dose cat exp. [28].

Among the children who were low-dose exposed to birch-pollen during the first 3 months of life, we observed that those who were sensitized to birch-pollen more often had detectable levels of IgG4 abs than the non-sensitized children. These findings could indicate that among low-dose-exposed children, IgG4 had a less immune-modulating effect, which is in line with the data of Böttcher et al. [20] concerning exp. during a 'normal' pollen season.

Exposure to high or low levels of birch-pollen during pregnancy led to diverse effects concerning the association between IgG4-ab response and IgE sensitization towards rBet v 1. Birch-pollen exp. during pregnancy resulted in a tendency towards increased sensitization to birch in the children of the high-dose-exposed group, as earlier reported [25]. Irrespective of the allergen dose, the number of children with detectable IgG4-antibodies was almost identical. However, more children of low-dose-exposed pregnant women and sensitized to

Table 2. Odds ratios for symptoms of atopy in children aged 4 to 5 years in Stockholm, in relation to IgE sensitization to birch and detectable IgG4 antibodies to birch

Clinical symptoms or history of atopic disease in the child	Numbers (n/N)	All children	
		OR (95% CI)	Adjusted OR (95% CI)*
Atopy†			
IgG4 ⁺ /IgE ⁺ ‡	24/31	3.8 (1.6–9.1)	3.9 (1.7–9.4)
IgG4 ⁺ /IgE ⁻	64/134	1.0 (0.7–1.5)	1.0 (0.7–1.5)
IgG4 ⁻ /IgE ⁺	61/75	4.9 (2.7–8.9)	4.8 (2.6–8.8)
IgG4 ⁻ /IgE ⁻	304/645	1.0	1.0
Bronchial asthma			
IgG4 ⁺ /IgE ⁺	9/31	1.3 (0.6–2.9)	1.4 (0.6–3.2)
IgG4 ⁺ /IgE ⁻	33/134	1.0 (0.7–1.6)	1.1 (0.7–1.7)
IgG4 ⁻ /IgE ⁺	18/75	1.0 (0.6–1.8)	1.0 (0.6–1.8)
IgG4 ⁻ /IgE ⁻	154/645	1.0	1.0
Allergic asthma			
IgG4 ⁺ /IgE ⁺	4/31	2.5 (0.8–7.5)	2.2 (0.7–6.7)
IgG4 ⁺ /IgE ⁻	13/134	1.8 (0.9–3.5)	1.6 (0.8–3.1)
IgG4 ⁻ /IgE ⁺	9/75	2.3 (1.1–5.0)	2.1 (0.9–4.6)
IgG4 ⁻ /IgE ⁻	36/645	1.0	1.0
Allergic rhinoconjunctivitis			
IgG4 ⁺ /IgE ⁺	12/31	5.4 (2.5–11.5)	5.5 (2.5–12.2)
IgG4 ⁺ /IgE ⁻	14/134	1.0 (0.5–1.8)	1.0 (0.5–1.9)
IgG4 ⁻ /IgE ⁺	40/75	9.7 (5.8–16.3)	9.4 (5.5–16.1)
IgG4 ⁻ /IgE ⁻	68/645	1.0	1.0
Atopic dermatitis			
IgG4 ⁺ /IgE ⁺	18/31	1.8 (0.9–3.7)	2.0 (0.9–4.2)
IgG4 ⁺ /IgE ⁻	56/134	0.9 (0.6–1.4)	1.0 (0.7–1.5)
IgG4 ⁻ /IgE ⁺	46/75	2.1 (1.3–3.4)	2.1 (1.2–3.4)
IgG4 ⁻ /IgE ⁻	280/645	1.0	1.0

*Odds ratio (95% confidence interval) adjusted for gender, birch pollen exposure postnatally or during pregnancy, maternal smoking, breastfeeding and pets in the household. If the confidence interval does not include 1.0, the result is significant at the $P < 0.05$ level.

†Clinical symptoms or history of atopic disease (bronchial asthma, rhinoconjunctivitis, and/or atopic dermatitis).

‡IgG4⁺ was defined as an IgG4-ab level ≥ 1.2 U/L against rBet v 1.

IgE⁺ was defined as skin prick test ≥ 3 mm against birch and/or an IgE-ab level ≥ 0.35 kU/L against birch and/or rBet v 1.

birch had detectable IgG4 abs than the non-sensitized children. The significance of this difference is difficult to interpret and previous publications concerning prenatal antigen exp. and IgE and IgG-abs responses have also been inconclusive. Thus, Jarret and Hall [19, 29] observed in the offspring of immunized female rats a selective antigen-specific suppression of the IgE-ab response while the primary IgG-ab response was augmented. The same effect has been suggested to occur in the children of mothers who undergo immunotherapy during pregnancy [21]. However, when women were exposed during late pregnancy to high or low doses of allergens through intake of food (cow's milk or hen's egg), their IgG ab concentrations were influenced by the diet, whereas the levels of IgG abs in cord blood were unaffected [22].

Secondly we studied the association between allergen-specific IgE and IgG4-ab responses and the development of atopic disease in children for the whole group of children. The development of allergic rhinoconjunctivitis seemed more common if the children were IgE sensitized, but showed a

negative IgG4 response. However, there was no association between increasing IgG4 levels and risk of developing symptoms of atopic disease. This suggests that the IgE response rather than IgG4 determines the allergic phenotype, and that IgG4 could exert an immune modulating effect on the clinical manifestations of atopic disease. In line with this, passive immunization with Ig from bee-keepers (in whom the anti-venom antibodies are known to be almost exclusively of the IgG4 ab class) has been shown to protect against venom-induced anaphylaxis. IgG abs were suggested to compete with IgE abs for allergens by blocking the binding sites, moderating the IgE reactivity [30].

Jenmalm et al. [31] have described that the levels of some IgG subclasses (not IgG4) in cord blood might be higher in children of atopic mothers, compared with children of non-atopic mothers. In our study, there was no difference in the prevalence of IgG4⁺ response in children with regard to maternal history of atopy (data not shown). However, we had no blood samples of the mothers at the time for delivery or cord blood from the babies; thus, the mothers own IgG4 levels and the passage of maternal IgG4 across the placenta are unknown. Besides, the data on various levels of IgG antibodies to inhalant allergens and association to development of atopy in the children are inconclusive [32, 33].

Could exp. to different inhalant allergens evoke diverse immune responses and biological effects? The association between exp. to food and inhalant allergens, IgE sensitization, IgG abs and development of atopic disease has been addressed in several publications the last few years [23]. Jenmalm et al. [31, 32] described how the IgG subclasses mature from birth up to 8 years in children exposed to inhalant allergens early in life. Several studies have shown that children exposed to high levels of reservoir allergens like cockroach, house dust mite (HDM) and mouse, are more likely than others to develop specific IgE sensitization [34, 35]. Furthermore, the IgG4-ab response to mites appears to be correlated to allergen exp. in the child's home [24]. Conversely, cat and dog allergens remain airborne and they can adhere to walls, carpets and clothing, facilitating the transport to schools and homes without a pet. Platts-Mills et al. [24, 36] found that exp. of children to moderate antigen levels resulted in IgE sensitization to the same allergen, while children exposed to very high levels of cat allergen were less likely to be sensitized to cat and more likely to have IgG4 abs to cat allergen. They hypothesized that sensitization to cat allergen Fel d 1 followed a more bell-shaped curve in relation to level of exp. to cat allergen [37]. In this study, we did not find the same effect by IgG4 on IgE sensitization and on atopic manifestation. The increased IgG4 response may not decrease the risk of sensitization to birch, but modulate the manifestations of atopic disease. Furthermore, different phenotypes could react in diverse ways and the development of disease may in some individuals be delayed. This might be elucidated by a future follow-up study when the children are older.

Could the divergence in the results from different studies of the effects of inhalant allergens be caused by methodological problems? One explanation could be difficulties comparing the levels of exp. to different allergens. Genetic factors [3] and other environmental factors such as endotoxin exp. might modulate an individual's response to allergens [8]. Further-

more, timing of exp. in relation to other exp.s and the age of the child are probably important [10, 25]. Assessment of exp. could be a problem when estimating, e.g., pet or mite allergen exp., because of difficulties in measuring all relevant environments over time. Furthermore, methods for laboratory analyses can differ between studies; in our study we measured specific IgG4 to rBet v 1. In some studies, total IgG has been measured and the IgG4⁺ levels extrapolated from these values. However, this procedure would yield less specific results.

Epidemiologic studies are sensitive to several types of bias [38]. For cross-sectional studies there is a risk of recall bias. The children in our study were investigated at the age of 5 years, thus, the parents probably still remember quite well several factors such as duration of breastfeeding, pets in the household and maternal smoking. Furthermore, adjustment for sibling number has been done and had no effects on the results. However, we have no objective data on infections in the first 5 years of life and such data are difficult to assess retrospectively. Our study, with a well-defined inhalant exp. during pregnancy and early infancy, serves as a unique model. However, the exp. in 1993 was unusually high. The mechanisms discussed above might possibly be less relevant following normal pollen seasons.

In conclusion, children exposed to high doses of birch-pollen during the first 3 months of life more often showed positive specific IgG4-ab response to rBet v 1. Thus, the effects of a short high-dose exp. (the pollen season of 1993) could be detected even after 5 years. IgE sensitization towards inhalant allergens appeared to be more important for the risk of atopic disease than the IgG4-ab levels. An immune-modulating effect of IgG4 on symptoms of allergic rhinoconjunctivitis is suggested in children sensitized to birch.

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