

Rapid Note

Birch pollen allergens fail to evoke IgG1 responses in non-atopic individuals

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(Received 2 January 1995; accepted 25 January 1995)

Key words: Birch pollen; Atopic disease; IgE; Allergen

Development of atopic diseases is associated with genetic susceptibility as well as with environmental factors. Production of IgE is regulated by two types of T-helper cells (Th), designated Th1 and Th2. These subpopulations are characterised by their production of defined sets of cytokines. Among these, the Th2-produced interleukin-4 (IL-4) is essential for IgE production to occur. As the Th1-produced interferon- γ counteracts the action of IL-4 these cytokines are key elements in determining the antibody response [1].

To better understand the atopic state, it is informative to compare the immunological reaction to allergens in atopic and non-atopic subjects. According to current concepts [2], when confronted with inhaled allergens in early childhood, the immune system reacts with both allergen-specific Th1 and Th2 responses. This will in turn result in IgG1 antibodies, induced by Th1 cells, and in IgG4/IgE induced by Th2 cells. Following continued allergen exposure, Th1 will come to dominate in non-atopic subjects with accompanying IgG1 antibodies while IgE disappears as a consequence of down-regulation of Th2. In atopic individuals, for unknown reasons, selection mechanisms will favour Th2, with resulting IgE production and declining IgG1 levels.

Here we attempted to test the validity of this notion by studying allergen-specific antibody production (IgG1 and IgG4/IgE) to birch pollen. Birch pollen was chosen (1) because it is a potent IgE sensitizer, and (2) because birch pollen is so widely spread in Northern Europe that all inhabitants in this area could be assumed to be exposed.

Sixty sera from adults with different atopy status were used. Twenty were from birch RAST-positive (birch-atopic group), 20 from birch RAST-negative (non-birch atopic group), and 20 from non-atopics (non-atopic group).

SDS-PAGE was performed in a Mini-Protean II Dual Slab Cell (Bio-Rad, Richmond, CA) according to the manufacturer's instructions. The separation slab gel was cast as a gradient (7.5–20%) on top of which the stacking gel was moulded (6%). The birch pollen extract (ALK Copenhagen, Denmark) was resuspended in a non-reducing sample buffer and 800 μ g of protein was loaded into a preparative well (74 $\times$ 1.0 mm). Low-molecular weight standard proteins (LMW, Electrophoresis Calibration Kit, Pharmacia) were loaded into a reference well. Subsequently, proteins were transferred onto nitrocellulose (Trans-blot NC membrane; pore size 0.45 μ m; Bio-Rad) according to the manufacturer's instructions. The membrane with standard proteins was stained with AuriDyeTM forte (Janssen Biotech, Olen, Belgium). The membrane with birch proteins was blocked for 60 min at 37°C with 0.5% Tween-20 in 0.05 M phosphate buffer, pH 4, also containing 0.15 M NaCl, 0.2% bovine serum albumin and 0.02% NaN₃. Next, the membrane was cut into narrow strips and incubated for 16–18 h with 0.5 ml of serum diluted 1:4 in blocking buffer. Following washing, the strips were subjected for 2 h to one of the following mouse mAbs: anti-human IgG1 (clone-6001, Sigma, St. Louis, MO) diluted 1:1000 in blocking buffer, or anti-human IgG4 (clone-6023, Sigma) (1:4000). For IgE determination rabbit anti-human IgE (MIAB, Uppsala, Sweden) (1:2000) was used. Next, alkaline-phosphatase (ALP)-conjugated rabbit anti-mouse IgG (Dakopatts, Glostrup, Denmark) diluted 1:4000 in blocking buffer was used for IgG1 and IgG4 analyses

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TABLE 1
PERCENTAGE OF SERA DETECTING THE MAJOR BIRCH ALLERGEN *Bet v I* IN IMMUNOBLOTTING

Source of sera	Major birch allergen 17 kDa (<i>Bet v I</i>)		
	IgE	IgG1	IgG4
Birch atopics (n = 20)	90	85	70
Non-birch atopics (n = 20)	0	0	0
Non-atopics (n = 20)	0	10	0

and goat anti-rabbit IgG-ALP (BioRad) (1:4000) was used for IgE analyses and was allowed to incubate 2 h. Finally, 1 ml of enzyme substrate (Alkaline Phosphatase Conjugate Substrate Kit, Bio-Rad) for 30 min. Sera that displayed a visible band at 17 kDa were scored as positive. In some experiments, recombinant *Bet v I* allergen (generously supplied by Dr. Lena Elfman, Pharmacia, Uppsala) was used in parallel with birch pollen extract to ascertain that the detected component in the crude extract was the major allergen.

Our immunoblotting results were clear-cut as shown in Table 1. In the birch-atopic group detectable levels of IgG1, IgG4 or IgE antibodies to the major birch allergen (*Bet v I*, a 17 kDa protein) [3] were frequently found. Out of 20 patients, 18 (90%) displayed IgE to *Bet v I*, 17 (85%) IgG1 and 14 (70%) IgG4. All sera from the non-birch atopic group were negative while 2 sera (10%) from the non-atopics had detectable IgG1 antibodies. Besides reaction with the major allergen, a few sera also reacted with other components. These reactions were preferentially found in the birch-atopic group (data not shown). The fact that the majority of the non-atopics as well as the non-birch atopic subjects failed to form IgG1 antibodies seems to suggest that the specific immune system disregards the allergens in these individuals despite exposure.

We favour the following scenario: normally when the mucosa of the airways encounters a large number of different inhaled substances, infectious agents like viruses and bacteria prompt a rapid action of the immune system. In contrast, non-infectious agents, like aeroallergens, normally should not elicit a specific immune response. However, in the susceptible individuals this discrimination occasionally fails giving rise to a specific immune response to a given allergen. It should be pointed out that even atopic individuals usually only react with a limited panel of allergen-specific IgE antibodies. Furthermore, our data seem to indicate that in atopic donors, Th1 and Th2 act side by side as reflected by presence of both specific IgG1 and IgG4/IgE.

The current concept that non-atopics have a Th1

response to inhaled allergens is based both on cellular and antibody response. Although the numbers of clones are small, allergen-specific Th1-type clones from non-allergic individuals have been described [4]. Analyses of sera from non-atopic donors, for the presence of allergen-specific IgG have generated conflicting results. Some investigators find allergen-specific IgG to mite allergens even in early infancy [5] while others studying grass pollen fail to detect specific IgG in non-atopics [6]. In the former case, crude extract of allergen was used in ELISA. We have recently applied both ELISA and immunoblotting for determination of mite-specific IgG. In our view, when using crude extract, immunoblotting is to be preferred as the molecular size of the recognised components could be determined [7]. Furthermore, when using solid-phase techniques care must be taken to exclude non-specific binding of IgG [8]. It is worthwhile considering that the concentration of IgG in serum is about 10^4 higher than that of IgE.

In conclusion, our data seem to indicate that non-allergic subjects normally raise no/low allergen-specific response while atopic subjects produce IgG1 as well as IgG4/IgE, indicating both Th1 and Th2 responses.

Acknowledgements

We thank Mrs. Rosa Bornstein for expert technical assistance. Financial support for this work was received from The Swedish Association against Asthma and Allergy, the Swedish Work Environmental Fund, the Swedish Medical Research Council (Grant 16x-105), Sigurd and Elsa Goljes Minne, Konsul Th. C. Bergh Foundation and the Karolinska Institute.

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