

IgG subclass antibody responses to birch pollen in sibling pairs discordant for atopy

Härfast B, van Hage-Hamsten M, Lilja G. IgG subclass antibody responses to birchpollen in sibling pairs discordant for atopy.
Pediatr Allergy Immunol 1998; 9: 208–214. © Munksgaard 1998

Birch pollen allergen specific IgG and IgE antibodies were analysed in the sera of fourteen sibling pairs discordant for atopy. In addition, eight unrelated children free of atopic disease were included in the study. The presence of Bet v 1 specific antibodies in the sera were analysed by an immunoblotting assay. All but one (13/14) of the atopic children had detectable anti-Bet v 1 antibodies of the IgG1 and IgG4 subclasses. The child lacking IgG1 and IgG4 antibodies to Bet v 1 was the only allergic child lacking IgE to Bet v 1. In contrast, only one of the non-atopic siblings (1/14) displayed detectable IgG1 antibodies to Bet v 1. Furthermore, among the non-atopic siblings none (0/14), had detectable IgG4 antibodies to Bet v 1. In the unrelated control group no detectable IgG1 or IgG4 anti-Bet v 1 were detected (0/8). Thus of the non-atopic children only one out of 22 children displayed IgG1 anti-Bet v 1 antibodies. Taken together, it appears that the non-atopic children in general have no/low allergen specific IgG to birch pollen.

**Bengt Härfast¹,
Marianne van Hage-Hamsten¹,
Gunnar Lilja²**

¹The Department of Laboratory Medicine, Division of Clinical Immunology, Karolinska Hospital,

²Sachs' Children's Hospital Stockholm, Sweden

Key words: birch pollen allergens; atopy; Bet v 1; allergen specific IgG antibodies; children; subclasses

Bengt Härfast, The Department of Laboratory Medicine, Division of Clinical Immunology, Karolinska Hospital, S-171 76 Stockholm, Sweden.

Accepted 5 October 1998

During the last few decades the prevalence of childhood atopy has increased dramatically (1, 2). Atopy is characterised by raised levels of allergen specific IgE antibodies (3). Although we now understand more of the cellular and molecular events that control IgE regulation, the reason for the increased incidence of atopy in the industrial part of the world remains unexplained. Recent studies (4, 5) suggest that sensitisation actually starts before birth since food and inhaled allergens by the mother could trigger the immune response of the fetus.

Normally, minute amounts of non-infectious agents fail to induce a specific immune response. In atopy, however, extremely small amounts of allergen elicit a specific IgE/Th2 response in susceptible individuals (6). This in turn causes the allergic manifestations. IL-4 plays a crucial role in the initiation of allergic disease as it provides the necessary signal for induction of IgE synthesis, while interferon- γ has the opposite effect. Atopic subjects produce in addition to IgE other classes and subclasses of allergen specific antibodies. Isotype switching is an important feature of the humoral response as it generates antibodies with the same specificity but with different effector functions (7). In non-atopic subjects, the involvement of the specific immune response to inhalant allergens is less clear. Some studies have found specific allergen antibodies (8) while other have failed to do so or found much lower

amounts (9–13). Here we scrutinised the antibody specific IgG responses to birch pollen, a widely spread and important inhalant allergen in Sweden, in sibling pairs discordant for atopy. The aim of the study was to gain some insight into the nature of the immune response elicited in non-atopics compared to that of atopics.

Subjects and methods

Subjects

Fourteen infants with a history of respiratory allergy - *i.e.*, bronchial asthma (BA; $n = 11$) and/or allergic rhino-conjunctivitis (RC; $n = 9$) against birch pollen were selected for the study and constitute the atopic group (Group A, Table 1). All had a positive SPT and/or a positive RAST against birch pollen. All atopic children except two (nos. 5 and 10; Table 1) had positive SPTs against other inhalant allergens and two (nos. 6 and 13) also gave positive SPTs against peanut. In the atopic group there were eleven boys and three girls with a mean age of 8.1 (range 3 to 11.75) years. Three infants were on regular medication with steroids for inhalation, none were on medication with oral steroids and none of the children were given immunotherapy.

In each sibling pair one of the children had manifest birch pollen allergy (Group A) whereas the

Table 1. Demographic data of the participating 14 pairs of siblings

No. of pair	Age (Yrs)	Sex	Atopic disease	Skin prick test ¹⁾	RAST birch class/kU/L
1 A	7.25	boy	BA, RC	birch, cat, dog	5/51
1 NA	1.5	boy	—	negative	0/< 0.35
2 A	5.75	boy	BA	birch, dog	2/2.5
2 NA	3.5	boy	—	negative	0/< 0.35
3 A	11.75	boy	RC	birch, timothy, mugworth	6/200
3 NA	5.25	boy	—	negative	0/< 0.35
4 A	9.5	boy	BA, RC, AD	birch, timothy, cat, horse, cladosporium	5/59
4 NA	7.5	girl	—	negative	0/< 0.35
5 A	9.25	boy	BA, RC	birch	5/59
5 NA	7.75	girl	—	negative	0/< 0.35
6 A	9.5	girl	BA, FA	birch, timothy, horse, peanut	3/6.5
6 NA	15.25	girl	—	negative	0/< 0.35
7 A	12.25	girl	RC, AD	birch, timothy	4/44
7 NA	2.25	girl	—	negative	0/< 0.35
8 A	9.75	boy	BA, RC	birch, timothy, cat, dog, horse	6/306
8 NA	1.0	girl	—	negative	0/< 0.35
9 A	4.0	boy	BA	birch, cat	6/188
9 NA	2.5	boy	—	negative	0/< 0.35
10 A	4.5	boy	BA	birch	6/107
10 NA	0.25	girl	—	negative	0/< 0.35
11 A	8.0	boy	BA, RC	birch, timothy, cat, dog	2/1.9
11 NA	4.5	boy	—	negative	0/< 0.35
12 A	8.5	girl	RC	ND	5/95
12 NA	6.5	girl	—	negative	0/< 0.35
13 A	5	boy	BA, AD, FA	birch, horse, cat, timothy, peanut	3/5.5
13 NA	1.5	girl	—	negative	0/< 0.35
14 A	9	boy	BA, RC	birch, horse, cat, dog, mugworth	4/46
14 NA	2	boy	—	negative	0/< 0.35

1) $\geq$ ++; diameter 3 mm and at least half the diameter of the histamine = 10 mg/ml.

A = atopic; NA = non-atopic; BA = bronchial asthma; RC = allergic rhinoconjunctivitis; AD = atopic dermatitis; FA = food allergy; ND = not done

brother/sister had no history of atopic disease (Group NA), past or present. There were six boys and eight girls who constituted the non-atopic group (Group NA, Table 1). They were negative in the RAST/SPT analysis against birch pollen and had a mean age of 4.4 (range 0.25 to 15.25 years). All "healthy" siblings except one (no. 6) were younger than the corresponding atopic sibling in the Group A.

An additional group of children, free from atopic diseases and without a positive history of atopic disease among first degree relatives, were also included in the study. This control group (Group C), unrelated to Groups A and NA, contained 8 children (2 boys and 6 girls) with a mean age of 8.1 (range 1.5 to 14) years and with negative RAST/SPT tests to birch pollen.

Study design

Both children in each sibling pair were simultaneously included in the study. This meant that the blood samples were always collected at the same

time within a pair of siblings to reduce the influence of inter- and intra-assay variation of the immunoglobulin analyses. The blood samples were collected outside the pollen season with the exception of sibling pairs nos. 1, 3, 4, 5 and 6.

Methods

Skin Prick Test (SPT): SPT against birch, timothy, mugworth, cat, dog, horse, *Dermatophagoides pteronyssinus* and *Cladosporium* was performed with Soluprick (10 HEP), ALK, Copenhagen, Denmark). A SPT was considered positive if the wheal was at least 3 mm in diameter and $\geq$ half the wheal of the positive reference (histamine 10 mg/mL).

RAST: IgE-antibodies in serum were analysed by Pharmacia-CAP system RAST FEIA (Pharmacia&Upjohn AB, Uppsala, Sweden) in accordance with the recommendations of the manufacturer. A RAST value $\geq$ 0.35 kU/L was considered positive.

SDS-PAGE and Immunoblotting: SDS-PAGE was performed in a Mini-Protean II Dual Slab Cell (Bio-Rad, Richmond, Calif., USA) according to the man-

ufacturer's instructions. The separation slab gel was cast as a gradient (7.5 to 20%) on top of which the stacking gel was moulded (6%). All sera were assessed against recombinant Bet v 1 (kindly provided by Dr Lena Elfman, Pharmacia & Upjohn AB). In addition, 12 sera were also tested for binding against birch pollen extract from ALK allergen. Either rBet v 1 (8 µg) or birch pollen extract (800 µg of protein) was resuspended in a non-reducing sample buffer and subsequently loaded into a preparative well (74 × 1.0mm). Low-molecular weight standard proteins (LMW, Electrophoresis Calibration kit, Pharmacia Upjohn) were loaded into a reference well. Subsequently, proteins were transferred onto nitrocellulose (Trans-blot NC membrane; pore size 0.45 µm; Bio-Rad) according to the manufacturer's instructions. The membrane with standard proteins was stained with AuriDye™ forte (Janssen Biotech NV, 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 to 18 hours with 0.5 ml of serum diluted 1:10 in blocking buffer. Following washing, the strips were subjected for two hours to one of the following mouse mabs: anti-human IgG1 (clone-6001, Sigma, St Louis, Missouri, USA), diluted 1:1000 in blocking buffer, or anti-human IgG4 (clone-6023, Sigma) (14000), or anti-human IgG2, (clone HP-6014, 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 and goat anti-rabbit IgG-ALP (Bio-Rad) (1:4000) was used for IgE analyses. The incubation time was two hours. Finally, one ml of enzyme substrate (Alkaline Phosphates Conjugate Substrate Kit, Bio-Rad) was incubated for 30 minutes. To determine total IgG, IgM and IgA ALP-conjugated rabbit anti-IgG (DAKO, Glostrup, Denmark) (1:1000), ALP-conjugated rabbit anti IgM (DAKO) (1:1000), and ALP-conjugated rabbit anti-IgA (DAKO) (1:1000), was used respectively. Sera that displayed a visible band at 17 kDa were scored as positive; +++ equaled strong band, ++ equaled medium strong band, + equaled weak band, - equaled no visible band.

Ethics

The study was approved by the Human Research Ethics Committee at Södersjukhuset, Stockholm, Sweden. Informed consent was given by the parents before the children entered the study.

Table 2. Immunoblot against Bet v 1 in atopic and non-atopic sibling-pairs

Subject No	IgE	IgG4	IgG1	IgG
1 A	+++ ¹⁾	+++	+++	+++
2 A	+	++	++	+++
3 A	++	+++	+++	+++
4 A	+++	+++	+++	+++
5 A	+++	+++	+++	+++
6 A	+	+	+	+
7 A	-	-	-	-
8 A	+++	+++	+++	+++
9 A	+++	++	+++	+++
10 A	+++	+++	+++	ND
11 A	+	+++	++	ND
12 A	+++	+++	+++	ND
13 A	+	+	+	ND
14 A	++	++	+++	ND
1 NA	-	-	-	-
2 NA	-	-	-	-
3 NA	-	-	-	-
4 NA	-	-	-	+
5 NA	-	-	+++	+++
6 NA	-	-	-	-
7 NA	-	-	-	-
8 NA	-	-	-	-
9 NA	-	-	-	-
10 NA	-	-	-	ND
11 NA	-	-	-	ND
12 NA	-	-	-	ND
13 NA	-	-	-	ND
14 NA	-	-	-	ND

1) +++ = strong band at 17 kDa; ++ = medium strong band; + = weak band, - = no visible band.

A = atopic, NA = non-atopic. ND = not done

Results

The presence of IgE-, IgG1- and IgG4-antibodies towards the major birch pollen allergen, Bet v 1, is presented in Table 2. Among the children in Group A 13/14 (93%) had detectable Bet v 1 specific antibodies of IgG1, IgG4 and/or IgE. However, the serum from one child (no. 7 A) showed no detectable antibodies towards Bet v 1 in spite of a positive birch-RAST (RAST class 4 (44 kU/L). Furthermore, three sera (nos. 2 A, 6 A and 13 A) displayed a weak IgE band which correlated with a low RAST class to birch (RAST class 2–3). In addition, the distribution of subjects with a positive or negative RAST to birch was the same as if they were grouped according to the IgG4 and IgG1 immunoblot results.

Among the children in the Group NA only one child (no. 5 NA; Fig. 1) of the 14 displayed a positive immunoblotting response. Interestingly, the immune response belonged to the IgG1-subclass while no IgG4 or IgE were detectable. In contrast, the atopic sibling (5A) scored positive in immunoblot for

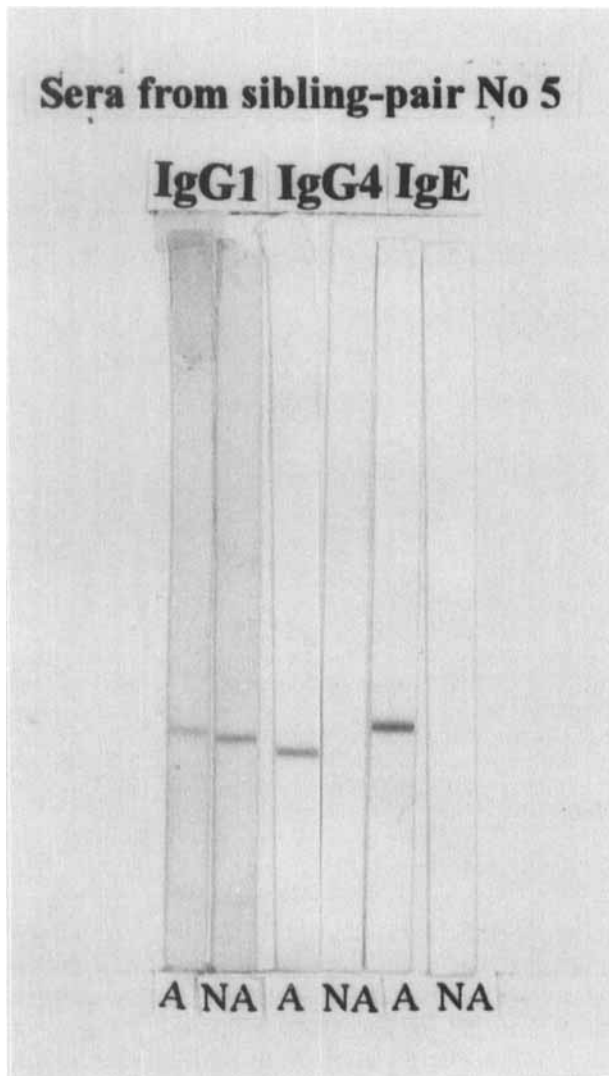


Fig. 1. SDS-PAGE immunoblotting of Bet v 1 with sera (diluted 1/10) from sibling pair no. 5; A = atopic, NA = non-atopic. The sera were tested for presence of antibodies specific for Bet v 1; The two left-hand lanes show assessment of Bet v 1 specific IgG1 antibodies; the two middle lanes show assessment of Bet v 1 specific IgG4 antibodies; the two right-hand lanes show assessment of Bet v 1 specific IgE antibodies.

IgE, IgG1 and IgG4 (Fig. 1). The immune reactivity against birch pollen extract from ALK in the two children in pair no. 5 was compared with that of two other sibling pairs (nos. 2 and 3) (Fig. 2). Child no. 5 NA was positive against a 17 kDa band as well as against a high molecular weight band. This is in contrast to the other two non-atopic siblings (nos. 2 NA and 3 NA), who were negative in immunoblotting (Fig. 2).

The immunoblotting results of the infants in Group C are presented in Fig. 3 and show that none of these children exhibit detectable levels of anti-Bet v 1 antibodies. If the children in Groups NA- and C were combined ($n = 22$), one child (no. 5; NA) out of 22 (5%) displayed anti-Bet v 1 positive

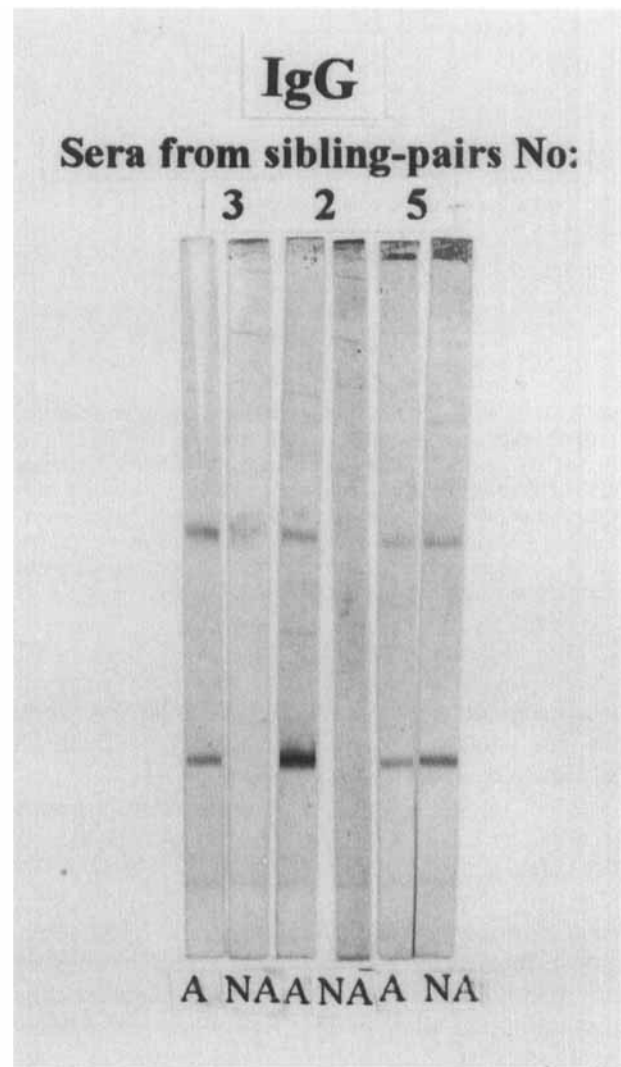


Fig. 2. SDS-PAGE immunoblotting of birch pollen extract with sera from sibling pairs nos. 3, 2 and 5; A = atopic, NA = non-atopic. Assessment of IgG antibodies binding components of birch pollen extract is shown. From left to right; 3 A, 3 NA, 2 A, 2 NA, 5 A, 5 NA.

reaction of the IgG1 subclass.

In order to get an indication of the magnitude of the IgE and IgG subclass binding in Western blot analysis, a serum (12 A, Fig. 4) from allergic children was diluted from 1/10 to 1/400 and assessed for specific antibodies to Bet v 1. For comparison, serum from the healthy sibling (12 NA) was also included at the lowest dilution (1/10). The intensity of the bands declined with increasing dilution but were visible in the serum from the allergic child in the highest dilution for IgG1, IgG2, IgG4 as well as for IgE. In contrast the serum from the non-atopic child displayed no bands.

In Figure 5, sera from an atopic (no. 8 A) and a healthy (no. 8 NA) sibling were assessed for specific anti-Bet v 1 antibodies. In the atopic serum strong

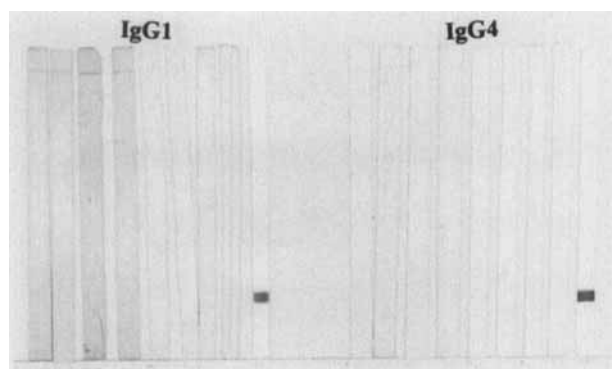


Fig. 3. SDS-PAGE immunoblotting of Bet v 1 with sera (diluted 1/10) from the eight non-atopic infants from the unrelated Group C. The left-hand part shows the assessment of Bet v 1 specific IgG1 antibodies. Sera from the eight non-atopic children analysed in the first eight lanes. In addition, serum from an atopic child, the last lane of the section, was included as a positive control. Assessment of Bet v 1 specific IgG4 antibodies (right-hand part) was performed as stated above for IgG1.

bands were detected for IgG, IgG1, IgG4, IgA while the IgM band was weak. The healthy child (no. 8 NA) was negative for all Ig classes.

Discussion

We have compared the allergen specific immune response in healthy and atopic infants. Our interest was mainly to elucidate whether or not healthy children mount an allergen specific IgG response comparable to that of their allergic counterparts. The results clearly show that the atopic children have raised a strong anti-Bet v 1 response of an IgG1, IgG2, and IgG4 nature while the non-atopic siblings failed to show detectable levels of allergen specific antibodies. As the group of atopic siblings were in general older than that of the non-atopic siblings we included an unrelated group of non-atopic children, age matched with the atopic group. None of the children in this non-atopic group displayed detectable Bet v 1 specific IgG antibodies. Our results are in agreement with Menz, *et al.* (14). Using ELISA and RAST, they found neither IgG1 nor IgG4 positive results among their nonatopic healthy controls. A majority of their RAST positive subjects also displayed IgG1 and IgG4 antibodies (14). Recently Jenmalm, *et al.* (15), using a sensitive ELISA, found that high levels of maternally derived anti-Bet v 1 IgG antibodies in cord blood were associated with atopy in the mother. In the present study, we decided to use Western blotting as we obtained more clear cut results with immunoblotting than with ELISA in a previous study (12). We have found in that study that adult non-atopic subjects failed to mount birch

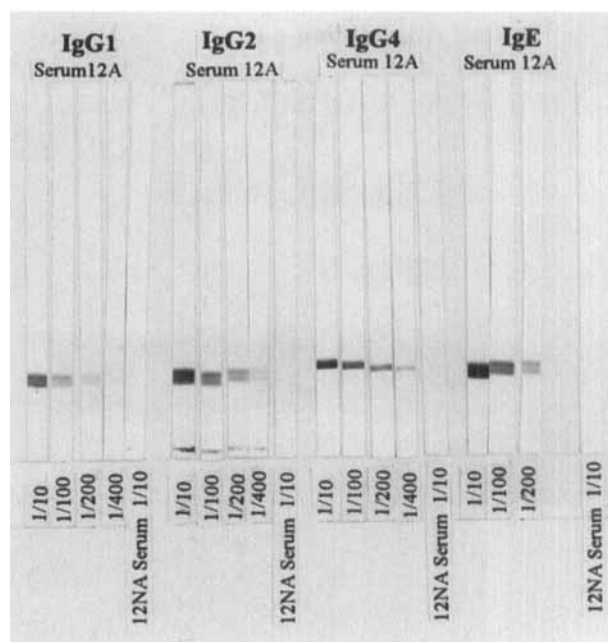


Fig. 4. SDS-PAGE immunoblotting of Bet v 1 with sera from sibling pair no. 12. A = atopic, NA = non-atopic. The sera was tested for the presence of Bet v 1 specific IgG1, IgG2, IgG4 and IgE antibodies as indicated. The atopic serum (A) was titrated from dilution 1/10–1/400. The non-atopic sera (NA) was used diluted 1/10.

pollen specific IgG1 and IgG4 antibody responses as analysed in immunoblotting using birch pollen extract (12). We chose to study the major allergen Bet v 1 in the present study, since the majority of birch pollen allergic subjects display IgE antibodies to this allergen (14). Interestingly, we found one serum from a healthy subject displaying strong IgG1 activity but lacking IgG4 and IgE against Bet v 1. We intend to re-examine the immune response of this subject and compare it to that of other non-atopics as well as atopics with respect to cytokine production and proliferation.

As pointed out, normal subjects stay healthy as they can inhale allergens without experiencing inflammation (16). No delayed type of hypersensitivity with a cellular inflammatory response to allergens is observed (16). Despite this fact, a common notion is that non-allergic children elicit the Th1-type of immune response resulting in production of IgG antibodies. This concept is supported by the cellular response showing that allergen specific T-cell clones derived from non-allergic subjects tend to be of Th1 type (17). However, it has never been demonstrated to our knowledge whether the clones are derived from secondary or primary responses, *i.e.* whether or not an *in vivo* response actually exists in the non-atopic subjects. Moreover, the allergen specific T-cell response of non-atopic subjects

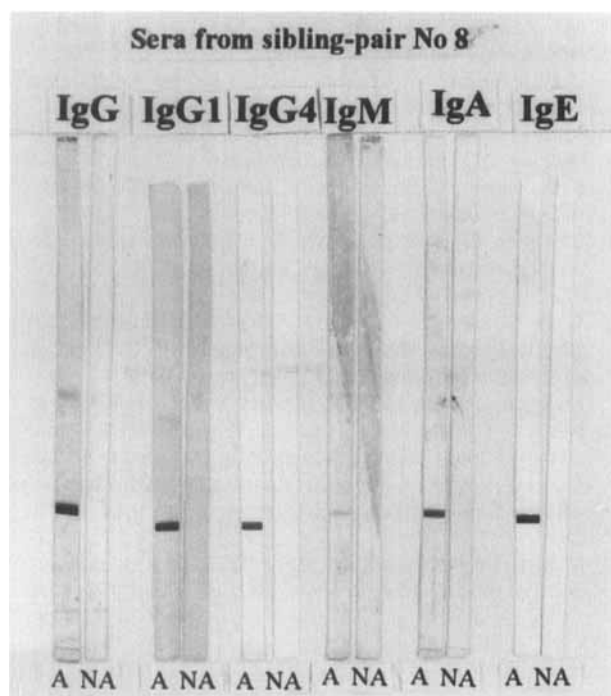


Fig. 5. SDS-PAGE immunoblotting of Bet v 1 with sera from sibling pair no 8. A = atopic, NA = non-atopic. Sera, diluted 1/10, was tested for presence of Bet v 1 specific IgG, IgG1, IgG4, IgM, IgA and IgE antibodies as indicated.

has been reported generally to be of a lower order of magnitude (10). As to the humoral response, several early studies clearly show that the IgG response is elevated in atopics (9–13). However, some authors claim that specific IgG antibodies against mites early in life occurs regardless of atopic status (8). The basis for this discrepancy (presence of IgG antibodies in both atopics and non-atopics) is unknown. The use of different techniques and of different allergens might be of importance. Some allergens which are present all year round, such as mite and cat allergens, may more frequently induce immune responses in non-atopics.

Several possibilities exist to account for this lack of detectable levels of anti-Bet v 1 IgG antibodies in the non-allergic subjects. First, it is possible that no specific immunological response to allergen is elicited in the healthy subjects to allergen. Second, it is possible that in the non-allergic individuals a specific immune response is elicited but fails to promote antibody formation. IL-4, the hallmark of Th2 response, has been shown to promote all IgG subclasses except IgG2 (18). A third possibility is that the healthy individuals have a transient humoral response to allergen which subsequently subsides. Fourth, the response may have the same nature as in the atopic subjects but at a much lower level which usually does not allow detection. Thus, the absence

of inflammation in non-atopics could be due to tolerance or lack of induction of a specific immune response.

As to the different subclasses of IgG, it is interesting to note that allergen specific anti-Bet v 1 antibodies are present in the sera of allergics. IgG2 is primarily considered to be directed to carbohydrate moieties. Moreover, the switch to IgG2 follows different routes than the other three IgG subclasses (18). Recently, it was shown that IL-4 in conjunction with anti-CD40 induced switching to IgG1, IgG3, and IgG4 but not IgG2 (18). In contrast, other cytokines like interferon- γ produced by Th1 cells, inhibit IgE production. Nevertheless, in allergic patients' sera, allergen specific IgG2 seems to be present at levels similar to the other IgG subclasses.

The data may also be explained by genetic influence on the capacity to respond to particular antigens. The influence of the human leukocyte antigens (HLA) on the immune response to allergens have previously been studied as these structures influence the range of antigens which can be presented to T-helper cells. For example, in a study of Marsh *et al.* (19) HLA-DR2 was found to be associated with the response to ragweed.

In summary, we have studied siblings, living in the same environment and therefore exposed to similar concentrations of allergen. In the children that remained healthy (excepting one child) with an IgG1 response we failed to trace signs of a Bet v 1 specific immune response as judged by lack of specific antibodies. In contrast, their atopic counterparts display Bet v 1 specific IgG1, IgG2, IgG4, IgA and IgM antibodies.

Acknowledgements

This work was supported by grants from: The Swedish Asthma and Allergy Association, The Swedish Council for Work Life Sciences, The Swedish Foundation for Health Care, Sciences and Allergy Research, The Elsa and Sigurd Goljes Memorial Fund, Consul Th. C. Berghs Foundation, The Södersjukhuset and the Karolinska Institute.

References

1. BURNET PGJ, CHINN S, RONA RJ. Has the prevalence of asthma increased in children? Evidence from the national study of health and growth 1973–1986. *Br Med J* 1990; 300: 1306–10.
2. ÅBERG N, HESSELMAR B, ÅBERG B, ERIKSSON B. Increase of asthma, allergic rhinitis and eczema in Swedish school-children between 1979 and 1991. *Clin Exp Allergy* 1995; 25: 815–9.
3. JOHANSSON SGO, BENNICHT H, BERG T. 3. Quantitative estimation of circulating IgE antibodies by radioallergosorbent test. *Int Arch Allergy Appl Immunol* 1971; 41: 443–51.
4. WARNER JA, JONES AC, MILES EA, WARNER JO. Prenatal sensitisation. *Pediatr Allergy Immunol* 1996; 7 Suppl 9: 98–101.

5. VAN DUREN-SCHMIDT K, PICHLER J, EBNER C, et al. Prenatal contact with inhalant allergens. *Pediatric Res* 1997; 41: 128–31.
6. HOLT PG. Immunoprophylaxis of atopy: light at the end of the tunnel? *Immunol Today* 1994; 15: 484–9.
7. VERCELLI D. Immunoglobulin E regulation in humans, 1989–1994. *Allergy* 1995; 50: 5–8.
8. RUIZ RG, PRICE JF, KEMENY DM. Specificity of ELISA for IgG subclass antibodies against inhalant antigens in early childhood. *Allergy* 1994; 49: 719–23.
9. TAME A, SAKIYAMA Y, KOBAYASHI I, TERA I, KOBAYASHI K. Differences in titres of IgE, IgG4 and other IgG subclass anti-Der p 2 antibodies in allergic and non-allergic patients measured with recombinant allergen. *Clin Exp Allergy* 1996; 26: 43–9.
10. GURKA G, OHMAN J JR, ROSENWASSER LJ. Allergen-specific human T cell clones: derivation, specificity, activation requirements. *J Allergy Clin Immunol* 1989; 83: 945–54.
11. JOHANSSON E, HÄRFAST B, JOHANSSON SGO, VAN Hage-HAMSTEN M. IgG1 and IgG4 antibody responses to the dust mite *Lepidoglyphus destructor* in a naturally exposed farming population. *Allergy* 1995; 50: 473–7.
12. HÄRFAST B, VAN Hage-HAMSTEN M, LILJA G. Birch pollen allergens fail to evoke IgG1 responses in non-atopic individuals. *Immunol Lett* 1995; 45: 223–4.
13. PLATTS-MILLS TAE. Local production of IgG, IgA and IgE antibodies in grass pollen hay fever. *J Immunol* 1979; 122: 2218–25.
14. MENZ G, DOLECEK C, SCHONHEIT-KENN U, et al. Serological and skin-test diagnosis of birch pollen allergy with recombinant Bet v I, the major birch pollen allergen. *Clin Exp Allergy* 1996; 26: 50–60.
15. JENMALM MC, HOLT PG, BJÖRKSTÉN B. Maternal influence on IgG subclass antibodies to Bet v 1 during first 18 months of life as detected with a sensitive ELISA. *Int Arch Allergy Immunol* 1997; 114: 175–184.
16. BORISCH L, ROSENWASSER L. Th1/TH2 lymphocytes: doubt some more. *J Allergy Clin Immunol* 1997; 99: 161–4.
17. WIERENGA EA, SNOEK M, DE GROOT C, CHRETIEN I, BOS JD, HENK M, JANSEN M, KAPSENBERG ML. Evidence for compartmentalization of functional subsets of CD4+ T lymphocytes in atopic patients. *J Immunol* 1990; 144: 4651–6.
18. FUJIEDA S, ZHANG K, SAXON A. IL-4 plus CD40 monoclonal antibody induces human B cells gamma subclass-specific isotype switch: switching to gamma 1, gamma 3, gamma 4, but not gamma 2. *J Immunol* 1995; 155: 2318–28.
19. MARSH DG, MEYERS DA, BIAS WB. The epidemiology and genetics of atopic allergy. *N Engl J Med* 1981; 305: 1551–59.