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A Sensitive ELISA to Detect IgG Subclass Antibodies to Bet v 1 in Infants

Key Words

IgG subclasses · Bet v 1 · ELISA · Enzyme amplification ·
Postnatal immune responses

Introduction

Epidemiological [1, 2] and prospective [3] studies strongly indicate that exposure to allergens early in life may have an impact on the incidence of allergy many years later. Prospective studies [4, 5] show that IgG responses to house dust mite in infancy are of the IgG1 subclass, while according to cross-sectional studies [6, 7], IgG4 responses appear later in life. Atopic children have higher titers of IgG antibodies to house dust mite than nonatopic children [5-7]. Little is known of the development of IgG subclass responses to other inhalant allergens. The aim of this study was to develop a sensitive ELISA based on enzyme amplification [8] for the detection of human IgG subclass antibodies to Bet v 1 in order to study the postnatal immune responses to an important seasonal allergen in detail.

Material and Methods

Serum Samples

Serum samples from 10 patients undergoing birch immunotherapy were screened for the presence of IgG1, IgG2, IgG3, and IgG4 antibodies to rBet v 1. The serum with the highest titers was used as an internal reference standard, and was calibrated according to a semiquantitative method modified from Sundin [9]. The postnatal immune responses to birch were then measured in 90 serum samples from 6-month-old infants participating in a prospective study of factors influencing the development of allergy. Thirty-six infants had an atopic mother, 22 had an atopic father, and 32 infants had no family history of atopy.

Conventional ELISA

Costar 3590 microtiter plates (Life Technologies AB, Täby, Sweden) were coated with rBet v 1 (a recombinant form of the major birch allergen, kindly provided by Prof. D. Kraft, Institute of General and Experimental Pathology, AKH, University of Vienna, Austria). Free binding sites were blocked with human serum albumin (HSA) (Chemicon, Stockholm, Sweden). Human serum samples or buffer without added serum (blanks) were added to duplicate wells, and then monoclonal antibodies to the human IgG subclasses: IgG (clone HP6064), IgG1 (clone HP6069), IgG2 (clone HP6014), IgG3 (clone HP6050) and IgG4 (clone HP6011) (Bio Zac AB, Järfälla, Sweden) were added. These clones were all recommended by WHO/IUIS [10]. Alkaline-phosphatase-conjugated rabbit anti-mouse IgG (Sigma-Aldrich, Stockholm, Sweden) was added, and *p*-nitrophenyl phosphate (pNPP) (Sigma-Aldrich) was used as substrate. The optical density (OD) was read at 405 nm. Values were expressed as arbitrary units (AU) deduced from the ODs of the reference serum curve after subtracting the blanks.

Enzyme Amplified ELISA

Coating, blocking and addition of human serum samples, monoclonal antibodies to the human IgG subclasses and alkaline-phosphatase-conjugated rabbit antimouse IgG were as described above. In the IgG and IgG1 assays, the ODs of HSA-coated wells were subtracted from the ODs of the serum samples (in the other assays, this was not necessary). For the substrate reaction, AMPAK kits (Dakopatts AB, Älvsjö, Sweden) were used. NADPH in diethanolamine buffer was allowed to react with the phosphatase for 20 min followed by 10 min incubation with amplifier solution. OD was measured at 492 nm. Values were expressed as AU deduced from the ODs of the reference serum curve, after subtracting the blanks.

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Table 1. Presence of IgG antibodies to birch in 90 serum samples of 6-month-old infants

	No of positive samples				
	IgG	IgG1	IgG2	IgG3	IgG4
Conventional ELISA	19	16	—	—	0
Enzyme amplified ELISA	75	58	4	11	6

The positive samples diluted out in parallel with the internal standard.

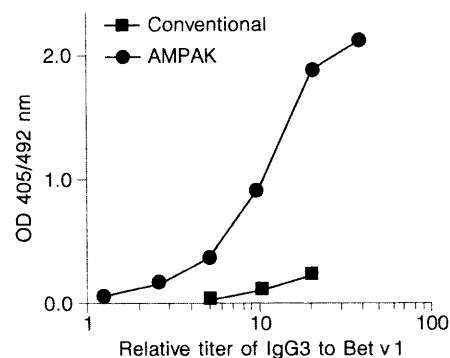


Fig. 1. Sensitivity of a conventional and an enzyme amplified ELISA (AMPAK in figure) for the detection of Bet-v-1-specific IgG3. The OD of the blank is subtracted from the OD of the sample, obtained from a patient undergoing immunotherapy.

Results and Discussion

The conventional ELISA could be optimized only for IgG, IgG1 and IgG4 antibodies against Bet v 1, since the levels of IgG2 and IgG3 antibodies were too low in all the serum samples from patients undergoing immunotherapy. This subclass restriction is common for allergens [11]. The titers of the internal standard were semiquantified as: IgG1 413 AU, IgG2 115 AU, IgG3 1.0 AU and IgG4 1845 AU (1 AU roughly corresponds to 1 ng/ml). Employing an ELISA combined with enzyme amplification, antibody assays could be established for all subclasses. Figure 1 shows representative dilution curves for IgG3 to Bet v 1. The intra-assay coefficients of variation were <5%. Interassay coefficients of variation ranged from 7 to 12%.

Ninety serum samples of 6-month-old infants were analyzed employing the different assays. The number of samples with detectable antibodies to Bet v 1 are shown in table 1. The proportion of samples with detectable antibodies to Bet v 1 and antibody levels were similar in infants with and without a family history of atopy. IgG antibodies to birch could be demonstrated in infants who had not yet experienced a postnatal birch pollen season. This may reflect intrauterine sensitization or exposure to antigens cross-reacting with birch, e.g. apple proteins. Trace amounts of maternal transplacentally transferred IgG antibodies can be present at 6 months of age [12]. Preliminary results, however, show that in some of the infants who had not yet experienced a pollen season, Bet v 1-specific IgG titers were higher in the 6-month sample than in cord serum.

In conclusion, a sensitive system enabling the detection of Bet-v-1-specific IgG subclasses in infancy has been developed. In 6-month-old children, IgG responses to birch were primarily of the IgG1 subclass although low titers of the other subclasses were detected in some samples.

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