Original Article

Change in the Pattern of IgE Reactivity to Timothy Grass and Birch Pollen Allergens Over a 20-Year Period

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Abstract. *Background*: Several studies have shown that the prevalence of allergy and allergen sensitization has increased in recent years. However, the changes in the pattern of IgE reactivity to individual allergens are mostly unknown.
Objective: The aim of this preliminary study was to assess the change in IgE reactivity profile to individual timothy grass and/or birch pollen allergens in sera from sensitized individuals randomly collected 20 years apart.
Methods: Serum samples from 51 sensitized individuals were obtained from 2 cross-sectional surveys performed in 1973 and 1994 using random samples from Vammala, Finland. The sera were analyzed for IgE reactivity to timothy grass and/or birch pollen extracts, recombinant (r)Phl p 1, 2, 5, 6, 7, 11, 12, native (n)Phl p 4, and rBet v 1, 2 and 4 by immunoassay (ImmunoCAP).
Results: The median (range) concentrations of IgE antibodies to timothy grass and birch pollen were higher in 1994 than in 1973 (6.47 [0.35 to >100] kU_A/L vs 1.53 [0.40–25.3] kU_A/L; $P = .0035$). The prevalence of IgE reactivity to some allergens was higher in 1994 than in 1973, particularly rPhl p 5 (52% vs 19%), rPhl p 6 (43% vs 12%), and rBet v 1 (100% vs 29%). There was a correlation between timothy grass pollen-specific serum IgE levels and the numbers of IgE reactivities to individual allergens ($\rho = 0.76$, $P < .001$).
Conclusions: The increase in specific IgE levels together with a possible increase in the prevalence of IgE reactivity to the major allergens Phl p 5 and Bet v 1 between 1973 and 1994 may have contributed to the increase in atopic conditions in Finland.

Key words: Birch pollen. IgE reactivity. ImmunoCAP. Recombinant allergens. Timothy grass pollen.

Resumen. *Antecedentes*: Varios estudios han demostrado que la prevalencia de alergia y sensibilizaciones a alérgenos ha aumentado en los últimos años. Sin embargo, los cambios en el patrón de la reactividad de la IgE frente a alérgenos individuales son prácticamente desconocidos.
Objetivo: El objetivo de este estudio preliminar es evaluar el cambio en el perfil de la reactividad de la IgE frente a alérgenos de hierba timotea y/o al polen de abedul encontrados en el suero de individuos sensibles reclutados al azar con 20 años de diferencia.
Métodos: Se recogieron muestras de suero de 51 individuos sensibles a través de 2 cuestionarios seccionales cruzados cumplimentados en 1973 y en 1994 como muestras aleatorias de Vammala, Finlandia. Las muestras de suero se analizaron para comprobar la existencia de reactividad en la IgE frente a extractos de hierba timotea y/o al polen de abedul, en la forma recombinante (r) Phl p 1, 2, 5, 6, 7, 11, 12, nativa (n) Phl p 4, y rBet v 1, 2 y 4 por inmunoensayo (ImmunoCAP).
Resultados: El rango de concentración de anticuerpos IgE frente a la hierba timotea y al polen de abedul resultó mayor en 1994 que en 1973 (6,47 [0,35 a >100] kU_A/L frente 1,53 [0,40-25,3] kU_A/L; P=0,0035). La prevalencia de reactividad de IgE a algunos alérgenos fue mayor en 1994 que en 1973, especialmente la rPhl p 5 (52% frente 19%), rPhl p 6 (43% frente 12%), y rBet v 1 (100% frente 29%). Existe una correlación positiva entre los niveles séricos IgE específica frente a hierba timotea y la IgE frente a alérgenos individuales.

Conclusiones: El aumento en los niveles de IgE específica junto con el posible aumento en la prevalencia de IgE frente a los alérgenos más comunes Phl p 5 y Bet v 1 entre los años 1973 y 1994 pueden haber contribuido al incremento de enfermedades atópicas en Finlandia.

Palabras clave: Polen de abedul. Reactividad de IgE. InmunoCAP. Alérgenos recombinantes. Polen de hierba timotea.

Introduction

Longitudinal epidemiological studies indicate that the prevalence of asthma and IgE-mediated allergy, as well as IgE sensitization to airborne allergens, has increased in developed countries in recent years [1-6]. The increased prevalence of allergy is thought to be associated with urbanization and improved standard of living. However, little is known about the change in IgE reactivity pattern to individual allergens over time.

In the present study, the IgE reactivity profile to recombinant and native timothy grass and birch pollen allergens was studied in sera from randomly selected subjects from 2 cross-sectional surveys performed 20 years apart.

Materials and Methods

Study Design

The design of the study from which the sera were obtained has been described previously [6]. Briefly, the field study was performed in Vammala, a small semi-urban community in south-west Finland with a relatively stable population size of about 16 000 residents. In 1973, 1 per 20 adult residents were randomly selected by computer and invited to participate. Twenty years later (1994), 50 men and 50 women in each 10-year age group were invited to participate. On both occasions the participation rate was 84%. Venous blood from the participants in the age range 15 to 54 years was collected in 1973 (n = 326) and 1994 (n = 319). Sera were stored at –20 °C prior to analysis of specific IgE antibodies in 1998 by immunoassay (ImmunoCAP 100, Phadia AB, Uppsala, Sweden). In the present study, only sera from subjects who were sensitized to timothy grass (n = 40) and/or birch pollen (n = 27) were included. These selected sera were retested in 2002 for total IgE and allergen-specific IgE antibodies, as described below. The 1973 and 1994 groups were matched for sex (proportion of females: 1973, 45%; 1994, 36%) and age (median [range], years: 1973, 24.9 [16.4–52.3]; 1994, 28.6 [15.6–45.0]). Six individuals in 1973 and 10 in 1994 were sensitized to both birch and timothy grass pollen. The study was approved by the Ethics Committee of the University Hospital, Tampere, Finland, and all participants gave informed consent.

Analysis of Specific and Total IgE

Eighteen sera from 1973 and 22 from 1994 obtained from individuals who were sensitized to timothy grass pollen (extract and/or any individual allergen) were analyzed for IgE reactivity to timothy grass (*Phleum pratense*) pollen extract and the individual allergens recombinant (r)Phl p 1, rPhl p 2, native (n)Phl p 4, rPhl p 5, rPhl p 6, rPhl p 7, rPhl p 11, and rPhl p 12 using the ImmunoCAP 100 immunoassay system. The corresponding number of sera from birch-sensitized individuals were 8 (1973) and 19 (1994). Those samples were analyzed for IgE reactivity to birch pollen (*Betula verrucosa*) extract and rBet v 1, rBet v 2, and rBet v 4. Total IgE was measured by immunoassay (immunoCAP 100). Specific IgE concentrations were expressed in terms of allergen-specific units (U_A) and concentrations above the measuring range of the assay (0.35–100 kU_A/L) were set as >100 kU_A/L.

Statistical Methods

Data for continuous variables are shown as the median (range). Mann-Whitney U test (two-tailed *P* value) and χ^2 test were used to evaluate differences between the results from samples obtained in 1973 and those obtained in 1994. Correlations were assessed using Spearman's rank correlation. *P* values of less than .05 were considered to indicate statistical significance.

Results

Total IgE Levels

The total IgE concentrations in the studied sera were similar in 1994 (153 [2.0–2270] kU/L) and 1973 (275 [11.7–3545] kU/L, *P* = .20). Total IgE levels for the timothy grass and birch pollen-sensitized subgroups are given in Tables 1 and 2.

Timothy Grass and Birch Pollen-Specific IgE Levels

The concentrations of specific serum IgE antibodies to grass and birch pollen (pooled data) were higher in

Table 1. Prevalence and Concentration of Specific IgE Antibodies to Individual Timothy Grass Pollen Allergens and Concentration of Total IgE*

Test		1973 (n=16)	1994 (n=21)	*P*
Timothy grass pollen extract	Prevalence	16 (100%)	21 (100%)	NA
	Concentration, kU_A/L	2.2 (0.40–25.3)	5.7 (0.35–>100)	.10
rPhl p 1	Prevalence	10 (62%)	18 (86%)	.10
	Concentration, kU_A/L	1.0 (0.39–8.1)	3.4 (0.37–91.0)	.30
rPhl p 2	Prevalence	4 (25%)	7 (33%)	.58
	Concentration, kU_A/L	0.64 (0.37–1.1)	3.8 (0.43–5.2)	.042
nPhl p 4	Prevalence	9 (56%)	14 (67%)	.52
	Concentration, kU_A/L	1.9 (0.36–7.6)	1.3 (0.35–14.5)	.87
rPhl p 5	Prevalence	3 (19%)	11 (52%)	.037
	Concentration, kU_A/L	7.5 (0.84–8.6)	3.7 (0.42–70.5)	.88
rPhl p 6	Prevalence	2 (12%)	9 (43%)	.045
	Concentration, kU_A/L	5.4 (5.1–5.8)	3.0 (0.43–17.4)	.22
rPhl p 7	Prevalence	0 (0%)	1 (5%)	.38
	Concentration, kU_A/L	NA	0.47 (NA–NA)	NA
rPhl p 11	Prevalence	3 (19%)	9 (43%)	.12
	Concentration, kU_A/L	0.73 (0.39–7.8)	2.7 (0.37–6.8)	> .9
rPhl p 12	Prevalence	3 (19%)	3 (14%)	.72
	Concentration, kU_A/L	0.39 (0.39–0.42)	0.44 (0.42–0.77)	.20
Total IgE	Concentration, kU/L	275.2 (11.7–3181.1)	194.6 (48.8–2270.4)	.66

*Prevalence is shown as number of individuals (%) and concentration as median (range). Criterion for inclusion in analysis was a positive result with the extract-based test or at least 1 of the component-based tests shown in the table.
NA indicates not available

Table 2. Prevalence and Concentration of Specific IgE Antibodies to Individual Birch Pollen Allergens and Concentration of Total IgE*

Test		1973 (n=7)	1994 (n=19)	*P*
Birch pollen extract	Prevalence	6 (86%)	19 (100%)	.27
	Concentration, kU_A/L	0.89 (0.42–5.3)	7.3 (0.74–>100)	.024
Bet v 1	Prevalence	2 (29%)	19 (100%)	<.001
	Concentration, kU_A/L	2.3 (0.66–3.9)	4.7 (0.39–>100)	NA
Bet v 2	Prevalence	2 (29%)	3 (16%)	.59
	Concentration, kU_A/L	0.37 (0.35–0.39)	0.62 (0.44–1.0)	NA
Bet v 4	Prevalence	0 (0%)	0 (0%)	NA
	Concentration, kU_A/L	NA	NA	NA
Total IgE	Concentration, kU/L	1361.3 (11.7–3544.7)	170.3 (2.5–2270.4)	.11

*Prevalence is shown as number of individuals (%) and concentration as median (range). Criterion for inclusion in analysis was a positive result with the extract-based test or at least 1 of the component-based tests shown in the table.
NA indicates not available

1994 than in 1973 (6.47 [0.35 to >100] kU_A/L vs 1.53 [0.40–25.3] kU_A/L; $P = .0035$), and similar differences were observed when timothy grass and birch pollen-specific IgE antibodies were studied separately (Tables 1 and 2).

The results of the 1998 and 2002 analyses of timothy grass and birch pollen-specific IgE antibodies were strongly correlated (timothy, $\rho = 0.93$, $P < .001$; birch, $\rho = 0.96$, $P < .001$). Nevertheless, 3 sera from 1973 and 1 from 1994 that were reported to be positive for IgE antibodies to birch (1 sample) or timothy grass (3 samples) in 1998 (0.5 – 2.6 kU_A/L) were negative for all pollen allergens when retested in 2002 and were therefore excluded from the prevalence estimations.

Reactivity to Individual Timothy Grass Pollen Allergens

In both 1973 and 1994 the most frequent IgE reactivity was to rPhl p 1 followed by nPhl p 4 (Table 1). IgE antibodies to several grass allergens, but especially to rPhl p 5 and rPhl p 6, were more prevalent in the sera from 1994 than in those from 1973. All sera with IgE reactivity to rPhl p 6 also contained IgE to rPhl p 5. The concentrations of specific IgE to rPhl p 2 were higher in the sera from 1994 than in those from 1973 (Table 1).

A strong correlation was observed between the concentration of timothy grass pollen-specific serum IgE and the number of IgE antibody reactivities to individual timothy grass pollen allergens ($\rho = 0.76$, $P < .001$).

Reactivity to Individual Birch Pollen Allergens

The prevalence of IgE reactivity to rBet v 1 was higher in 1994 than in 1973 (Table 2). In 1994, all birch pollen-sensitized individuals had IgE antibodies to rBet v 1. No relevant difference was observed for the Bet v 2 sensitization and no individuals had IgE antibodies to rBet v 4.

Discussion

The results of the present study comparing sera collected in 1973 and 1994 indicate that the concentrations of grass and birch pollen-specific IgE antibodies increased in sensitized Finnish subjects over a 20-year period. Elevated serum concentration of timothy grass pollen-specific IgE was associated with more IgE reactivities to individual allergens, as reported previously [7-9].

The study included sera stored at –20 °C for 8 or 29 years. There was a very strong correlation between the 1998 and 2002 analyses of the same serum samples, indicating high stability of the samples. IgE is normally very stable at –20 °C [10] and it has recently been observed that serum samples can be stored for at least 17 years without any apparent degradation of IgE measured by ImmunoCAP immunoassay [11].

IgE reactivity to rPhl p 5 and rPhl p 6 in particular was more common in 1994 than in 1973 in timothy grass-sensitized subjects. Phl p 6 has about 60 % sequence homology with Phl p 5 and they have cross-reactive potential [12]. IgE reactivity to rPhl p 1 and nPhl p 4 were the most common reactivities in both 1973 and 1994. All 4 of these allergens can be considered as major timothy grass pollen allergens [7]. However, the prevalence of IgE reactivity to individual grass allergens may vary in allergic patients from different countries [13]. Generally, the prevalences in the present study were somewhat lower than previously published data [7, 13].

All birch pollen-sensitized individuals from 1994 had IgE antibodies to the major birch pollen allergen Bet v 1. In 1973, only a minority were Bet v 1 sensitized. The observed prevalence of Bet v 1 sensitization in 1994 is consistent with other studies showing that more than 95 % of birch-pollen allergic patients living in northern and central Europe have serum IgE antibodies to Bet v 1 [14]. The observed prevalences of Bet v 2 sensitization of 16% to 29 % are higher than previously reported for northern Europe [14].

The difference in the sensitization patterns between this and other studies might be due to the inclusion criteria. In the present study, the subjects were selected from 2 random population samples due to their IgE sensitization to timothy grass and/or birch pollen, not due to symptoms.

IgE reactivities to Phl p 1, Phl p 5, and Bet v 1 have been suggested to be markers of original sensitization to grass (Phl p 1 and Phl p 5) and birch (Bet v 1) pollen and associated with clinical symptoms [15]. IgE reactivity to other pollen allergens such as profilins (eg, Phl p 12 and Bet v 2) and polcalcins (Phl p 7 and Bet v 4) may be due to cross-reactive IgE antibodies induced in response to homologous allergens from distantly related plants [15]. The observation that IgE reactivity to rPhl p 5 and rPhl p 6 was more frequent in grass-sensitized individuals in 1994 than 20 years earlier is notable. The overall grass pollen exposure in Western Europe is unlikely to have changed much in 20 years [16]. We observed a similar difference regarding Phl p 5/Phl p 6 sensitization between Russian and Finnish Karelia in a study performed in 1998, indicating an association with lifestyle and standard of living [8]. In the same study, an association between self-reported hay fever and sensitization to Phl p 5 was also observed.

We speculate that changes in sensitization patterns towards more Bet v 1 and Phl p 5/Phl p 6 sensitization and increased specific IgE levels might have contributed to the increase in atopic conditions reported for Finland and many other developed countries in recent years [1-3]. However, the available sera from birch- and grass-sensitized individuals were limited in the 1973 and 1994 surveys. The results from the statistical tests should therefore be interpreted with caution, and generalization of the results should be avoided unless the *P* values are

very low. Our study should therefore be considered as preliminary and should be followed up in future longitudinal studies with larger population samples.

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