

Dissociation of allergen-specific IgE and IgA responses in sera and tears of pollen-allergic patients: A study performed with purified recombinant pollen allergens

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Background: Trees and grass pollen allergens represent potent elicitors of allergic rhinoconjunctivitis and asthma. Little is known regarding the presence of allergen-specific IgA antibodies in sera and tears and their association with IgE responses in patients with allergic conjunctivitis.

Objective: The purpose of this study was to compare the specificities of IgE and IgA antibodies in sera and tears of pollen-allergic patients with conjunctivitis by using purified recombinant pollen allergens.

Methods: Sera and tears collected from 23 pollen-allergic and from 23 nonatopic individuals were analyzed for IgE and IgA reactivity to nitrocellulose-blotted birch and timothy grass pollen extracts. In addition, we determined the specificities of IgE, IgG₁₋₄, and IgA antibodies with use of a panel of purified recombinant pollen allergens (timothy grass: rPhl p 1, rPhl p 2, rPhl p 5; birch: rBet v 1, rBet v 2) in serum and tear samples by immunoblotting and ELISA. Statistical analyses of data were performed by *t* test and Mann Whitney *U* test.

Results: Serum and tears of many of the pollen-allergic individuals with conjunctivitis exhibited specificity for the very same pollen allergens. No allergen-specific IgE antibodies were detected in tears of nonatopic individuals. IgA antibodies in sera and tears of patients with allergic conjunctivitis were mainly directed against nonallergenic moieties and showed specificities that were significantly different from those of IgE antibodies.

Conclusion: The dissociation of IgE and IgA responses and the lack of allergen-specific IgA antibodies in mucosal secretions (eg, tears) may contribute to allergic manifestations in target organs of atopy. Induction of allergen-specific IgA antibodies

may hence be considered as a promising strategy for the treatment of mucosal forms of atopy. (*J Allergy Clin Immunol* 2000;105:803-13.)

Key words: Recombinant pollen allergens, allergic conjunctivitis, allergen-specific IgA and IgE, mucosal atopy

Seasonal allergic conjunctivitis represents one of the most frequent symptoms in patients with tree and grass pollinosis.¹ There is considerable evidence that IgE antibodies in tears are directed to similar allergen sources as IgE antibodies that are present in serum and that the symptoms of ocular allergy are associated with the levels of allergen-specific IgE in tears.²⁻⁴ Other markers indicative for ocular allergy are the mast cell–derived protease, tryptase,⁵ substance P,⁶ eosinophilic cationic protein,⁷ and increased levels of intercellular adhesion molecule-1 on conjunctival epithelial cells.^{8,9} The more chronic forms of ocular allergy (ie, atopic keratoconjunctivitis, atopic blepharoconjunctivitis, vernal keratoconjunctivitis, and giant papillary conjunctivitis) are characterized by the infiltration of T cells belonging to the type 2 T helper (T_H2) subset¹⁰ and the preferential secretion of T_H2-like cytokines (eg, IL-4, IL-5).¹¹

With use of natural allergen extracts several investigators have demonstrated the presence of IgE antibodies in the tears of patients with allergic conjunctivitis.²⁻⁴ By immunoblotting, some evidence was provided that the specificities of the IgE in tears may be similar to those of serum IgE, although it was suggested that tear-specific IgE might be produced locally.⁴ Nothing is, however, known regarding the specificities of tear-specific IgA and IgG antibodies because previous investigations were performed with allergen extracts instead of with purified and defined allergens.^{4,12}

Allergen-specific IgA antibodies in mucosal secretions might have influence on local allergic manifestations and perhaps even protect against allergy if they were directed to allergen epitopes recognized by IgE. Therefore several attempts have been made to induce protective allergen-specific IgA responses by oral immunotherapy with allergen extracts packed into enterocoated capsules. Some of the oral immunotherapy

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Abbreviations used

ABTS: 2,2'-Azine-bis(3-ethylbenzthiazoline-6-sulfonic acid)
 cDNA: Complementary DNA
 RAST: Radioallergosorbent test
 T_H2: Type 2 T helper cell

studies demonstrated clinical efficacy,¹³⁻¹⁵ whereas others reported that oral immunotherapy is ineffective.¹⁶ The evaluation of immunologic changes (ie, induction or changes of allergen-specific IgA) during oral immunotherapy studies has, however, been hampered by the lack of defined allergens for analysis.¹⁵

In recent years a considerable number of pure recombinant allergens have become available that closely resemble the immunologic and biologic properties of their natural counterparts and thus can be used for component-resolved allergy diagnosis.¹⁷⁻¹⁹

Here we used a panel of purified recombinant birch and timothy grass pollen allergens to study the specificities of IgE and IgA antibodies in serum and tears of patients with allergic conjunctivitis. Serum and tear samples were collected from a group of patients with allergic conjunctivitis and, for control purposes, from patients with predominantly allergic rhinitis as well as from nonatopic individuals and were analyzed for the presence of allergen-specific IgE and IgA antibodies. Our finding that IgE and IgA responses in patients with allergic conjunctivitis are dissociated is discussed in view of therapeutic strategies that aim at the induction of "blocking" allergen-specific IgA antibodies in mucosal secretions.

METHODS**Characterization of allergic patients and collection of sera and tears**

Twenty-three patients allergic to grass pollen or birch pollen were selected according to a case history indicative of seasonal pollen allergy (3 with predominantly allergic rhinitis, 20 with seasonal allergic conjunctivitis), positive skin reactions to birch and timothy grass pollen extract, and the demonstration of specific IgE antibodies to birch and timothy grass pollen extract (CAP-RAST measurements, Pharmacia & Upjohn, Uppsala, Sweden) as well as to recombinant pollen allergens.^{20,21} Twenty-three nonatopic individuals who were negative according to the above criteria were included for control purposes. The demographic and clinical characterization of the individuals is summarized in Table I. Sera were collected by venipuncture and stored at -20°C until use, and tear samples were obtained by soaking Schirmer test strips. The strips were extracted in equal amounts of PBS containing 10% BSA and stored at -20°C until use.

Natural pollen extracts and purified recombinant allergens

Pollen from timothy grass (*Phleum pratense*) and birch (*Betula verrucosa*) was purchased from Allergon (Välinge, Sweden). Pollen extracts were prepared by homogenization of 1 g pollen in 25 mL of distilled water containing 5 mmol/L phenylmethylsulfonyl fluoride with an ultraturax (Ika, Heidelberg, Germany).²² Proteins were further extracted under continuous shaking at 4°C for 1 hour. Extracts were then centrifuged in an SS 34 rotor at 18,000 revolu-

tions/min for 30 minutes to remove insoluble particles. One-milliliter aliquots of the supernatants were frozen in liquid nitrogen, lyophilized, and stored at -20°C until use. Lyophilized extracts were analyzed by SDS-PAGE²³ and Coomassie brilliant blue staining²⁴ (Bio-Rad, Richmond, Calif) to estimate the quality and quantity of proteins. Purified recombinant timothy grass pollen allergens (rPhl p 1,^{25,26} rPhl p 2,²⁷ rPhl p 5²⁸) and recombinant birch pollen allergens (rBet v 1,^{29,30} rBet v 2³¹) that had been expressed in *Escherichia coli* were obtained from BIOMAY (Linz, Austria). All recombinant allergens were shown in previous studies to have comparable immunologic and biologic activities to their natural counterparts.

IgE and IgA immunoblotting

Grass pollen extracts, birch pollen extracts (approximately 200 µg/cm gel) and recombinant allergens (rPhl p 1, rPhl p 2, rPhl p 5, rBet v 1, rBet v 2) (approximately 5 µg/cm gel) were separated by 12.5% preparative SDS-PAGE²³ and blotted onto nitrocellulose.³² Sera and tears were diluted 1:10 in buffer G (50 mmol/L sodium phosphate, pH 7.5, 0.5% vol/vol Tween 20, 0.5% wt/vol BSA, 0.05% wt/vol sodium azide) for IgE immunoblotting. IgA immunoblots were performed with 1:100 diluted serum or tear samples. Bound IgE and IgA antibodies were detected with iodine 125-labeled antihuman IgE antibodies (Pharmacia) and a ¹²⁵I-labeled mouse monoclonal antihuman IgA antibody that recognizes an epitope on IgA₁ and IgA₂ (MH14M, CLB, Amsterdam, The Netherlands), respectively. Bound ¹²⁵I-labeled antibodies were visualized by autoradiography with use of Kodak X-Omat-S films and intensifying screens (Kodak, Heidelberg, Germany). Serum as well as tear assays were run separately for each extract and component by including samples with already-established reactivity profiles (positive control) and buffer without addition of serum or tears (negative control). Reproducibility and reliability of the immunoblotting technique were confirmed by repeating the experiments with similar outcomes.

IgE and IgG₁₋₄ subclass ELISA

ELISA plates (Nunc Maxisorb, Roskilde, Denmark) were coated with purified recombinant allergens (rPhl p 1, rPhl p 2, rPhl p 5, rBet v 1, rBet v 2) dissolved at a concentration of 10 µg/mL in PBS at 4°C overnight. The plates were washed two times with buffer A (PBS, 0.05% Tween 20) and blocked with PBS containing 0.5% BSA, 0.05% Tween 20 at room temperature for 2.5 hours. Patient sera and tears were diluted in PBS, 0.5% BSA, 0.05% Tween 20 (1:5 for IgE and 1:50 for IgG₁, IgG₂, IgG₃, IgG₄ detection) and incubated with plate-bound allergens at 4°C overnight. Plates were washed 5 times with buffer A and bound human Igs were detected with mouse mAbs (mαhuIgE, mαhuIgG₁, mαhuIgG₂, mαhuIgG₃, mαhuIgG₄; Pharmingen, San Diego, Calif; mαhuIgA: CLB) diluted 1:1000 in PBS, 0.5% BSA, 0.05% Tween 20 overnight at room temperature. The plates were again washed 5 times with buffer A and incubated for 30 minutes at 37°C and 30 minutes at 4°C with a horseradish peroxidase-coupled sheep antimouse Ig antiserum (Amersham) diluted 1:2000 in PBS, 0.5% BSA, 0.05% Tween 20. After the plates were washed 5 times with buffer A, the color reaction was started with 2,2'-azinebis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) solution (60 mmol/L citric acid, 77 mmol/L sodium phosphate bihydrate, 1.7 mmol/L ABTS [Sigma, St Louis, Mo], 3 mmol/L hydrogen peroxide). The plates were incubated in the dark for 30 minutes at room temperature, and the color reaction was stopped by addition of 0.32% sodium fluoride. Extinctions (OD 450 to 405 nm) were measured with an ELISA reader (Dynatech, Denkendorf, Germany). Results were obtained as duplicate determinations and are displayed as mean OD with an error of less

TABLE I. Demographic and clinical characterization of allergic patients and nonatopic individuals

Patient No.	Sex	Age (y)	Symptoms										
			Sensitized to		Rhinitis			Conjunctivitis			Asthma		
					+/-	For years	Period	+/-	For years	Period	+/-	For years	Period
1	F	29	+	—	+	17	5-8	—			—		
2	F	28	—	+	+	18	5, 6	—			—		
3	F	43	—	+	+	28	5-7	—			—		
4	M	35	—	+	+	23	4-6	+	23	4-6	—		
5	F	37	+	+	+	19	4-6	+	19	4-6	—		
6	F	25	—	+	+	5	5-8	+	5	5-8	—		
7	M	29	+	—	+	13	5-7	+	13	5-7	—		
8	M	40	—	+	+	5	5, 6	+	5	5, 6	—		
9	M	35	—	+	+	23	5-9	+	23	5-9	—		
10	M	27	+	—	+	1	3, 4	+	1	3, 4	—		
11	M	31	—	+	+	14	5-7	+	14	5-7	—		
12	M	36	+	+	+	24	5, 6	+	24	5, 6	—		
13	F	38	+	+	+	19	5, 6	+	19	5, 6	—		
14	F	30	—	+	+	15	5-8	+	15	5-8	—		
15	M	32	+	+	+	21	5, 6	+	21	5, 6	—		
16	F	29	—	+	+	3	6-8	+	3	6-8	—		
17	M	28	+	+	+	20	4-7	+	20	4-7	—		
18	M	38	—	+	+	17	5-7	+	17	5-7	—		
19	F	65	+	—	+	30	4-7	+	30	4-7	—		
20	M	33	—	+	+	9	5-7	+	9	5-7	—		
21	M	37	—	+	+	23	5-7	+	23	5-7	+	23	5-7
22	F	28	—	+	+	10	5-7	+	10	5-7	+	6	6
23	M	42	+	+	+	16	5, 6	+	16	5, 6	+	16	5, 6

Sex, age, and clinical sensitization to birch and grass pollen are indicated. Symptoms (rhinitis, conjunctivitis, asthma) and their duration and occurrence during certain months of the year are displayed.

than 5%. Values for IgE are shown after subtraction of the mean OD (cutoff level) determined for sera from 5 nonatopic individuals who were negative in radioallergosorbent (RAST) and skin prick tests. Assays were run separately for each extract and component by including samples with already-established reactivity profile (positive control) and buffer without addition of serum/tears (negative control). Reproducibility and reliability of the ELISA technique were confirmed by repeating the experiments with similar outcome.

Statistical analysis of data

Statistical analyses of serum IgE and IgA reactivities in the atopic versus nonatopic group of individuals were performed by *t* test with use of MS Excel 97 analytic functions (Microsoft, Redmond, Wash). Statistical analyses of IgE versus IgA reactivities to given allergens were done with the nonparametric Mann-Whitney *U* test.

RESULTS

Dissociation of IgE and IgG subclass reactivities to recombinant pollen allergens in sera from pollen-allergic patients

The study population consisted of 20 patients with seasonal allergic conjunctivitis and 3 persons with predominantly allergic rhinitis (Table I). Nineteen of the 20 conjunctivitis patients had Western blot–detectable timothy grass pollen–specific IgE and 10 reacted with natural birch pollen allergens (Fig 1). IgE responses to recombinant timothy grass pollen allergens were found in most

of the grass pollen–allergic patients. Twenty-one of 23 patients reacted with rPhl p 1, 18 of 23 with rPhl p 2, and 22 of 23 with rPhl p 5 (Table II). IgE reactivity to rBet v 1 was detected in all 19 sera from birch pollen–allergic patients and rBet v 2 (birch profilin) was recognized in 8 patients (Table II).

As in previous studies²⁵ we found no association of IgE reactivities to a given recombinant allergen with any of the 4 IgG subclass (IgG₁₋₄) reactivities (data not shown).

Tears of patients with allergic conjunctivitis contain IgE antibodies to recombinant tree and grass pollen allergens

Although the 3 patients who predominantly had allergic rhinitis had timothy grass– or birch pollen–specific serum IgE antibodies as well as serum IgE directed to recombinant allergens (patients 1 to 3, Figs 1 and 2), we could not detect allergen-specific IgE in the tears of these patients (patients 1 to 3, Table II). By contrast, we found IgE antibodies with specificity to nitrocellulose blotted natural timothy grass or birch pollen extract (patients 4, 5, 8, 13, 16, 17, 19, and 23, Fig 2, A and B) as well as to recombinant timothy grass or birch pollen allergens (patients 4, 5, 8, 13, 14, 16, 17, 19, and 23, Table II) in tears of several patients with allergic conjunctivitis. Thus

TABLE II. Serologic characterization of allergic patients and nonatopic individuals

Patient No.	rPhl p 1				rPhl p 2				rPhl p 5			
	Serum IgE	Serum IgA	Tear IgE	Tear IgA	Serum IgE	Serum IgA	Tear IgE	Tear IgA	Serum IgE	Serum IgA	Tear IgE	Tear IgA
1	0.004	+/-			0.006				0.015			
2					0.028				0.184			
3	0.012				0.045				0.199	+		+/-
4	0.022			+/-	0.035				0.117	+/-	++	+/-
5	0.344	+/-			0.092				0.572		+	
6	0.491				0.013				1.093			
7	0.012				0.011				0.012			
8	0.977	++	++						1.961	+/-	++	
9	0.081				0.029				0.176			
10									0.002	+/-		
11	0.037	+/-			0.019				0.173		+	
12	0.061								0.226			
13	0.023	++			0.022				0.014			
14	0.027	+/-			0.012				0.169			
15	0.454	+/-							0.405			
16	1.279	+/-	++		0.035				2.156		++	
17	1.811	+/-	++						2.290		++	
18	0.401	+/-			0.109				1.376			
19	0.004	+/-			0.006							
20	0.318	++		+/-	0.094				1.219			
21	0.142	+			0.036				0.390			
22	0.271	+			0.083				0.110	+/-		
23	1.811	++	++		2.297		++		2.448		++	
24		+/-										
25		+/-										
26		+										
27												
28		+/-										
29												
30												
31												
32												
33		+/-										
34		+/-										
35										+/-		
36												
37												
38		+/-										
39												
40												
41												
42												
43		+/-										
44		+/-										
45		+/-										
46												

Sera from 23 pollen-allergic (patients 1-23) and 23 nonatopic individuals (patients 24-46) were analyzed for timothy grass pollen- and birch pollen-specific IgE (immunoblot), IgE, and IgA reactivity to purified timothy grass pollen allergens (rPhl p 1, rPhl p 2, rPhl p 5) and to purified birch pollen allergens (rBet v 1, rBet v 2) by ELISA. Reactivity to blotted allergens is indicated as strong (++), positive (+), or weakly positive (+/-). Lack of IgE binding to blotted allergen or IgE OD values below cutoff value were not displayed.

tears from 9 of the 20 patients with allergic conjunctivitis contained IgE antibodies to recombinant timothy grass or birch pollen allergens. No allergen-specific IgE antibodies were detected in tears of the 23 nonatopic individuals (Table II).

IgE antibodies in sera and tears of patients with seasonal allergic conjunctivitis are mostly directed to the same allergens

Because in previous studies total allergen extracts were used instead of purified allergens, it was not possible to

Bet v 1				Bet v 2			
Serum IgE	Serum IgA	Tear IgE	Tear IgA	Serum IgE	Serum IgA	Tear IgE	Tear IgA
0.052							
0.001							
0.023				0.02			
0.662		+		0.025			
0.038				0.007			
0.057				0.008			
					+/-		
0.012					+/-		
0.057							
					+/-		
0.026							
0.23		+/-					
0.013				0.005		+	
0.042							
0.021				0.016			
0.095		+/-		0.003		+/-	
0.004							
0.349		++					
0.027							
0.012				0.008	+/-		
0.063							
					++		
					+/-		
					+/-		
					+/-		
					+/-		

compare the specificities of serum IgE and tear IgE at a component-resolved level.²⁻⁴ We therefore used a panel of 3 recombinant timothy grass and 2 recombinant birch pollen allergens to detect the specificities of serum- and tear-derived IgE antibodies (Table II). In most cases we found

that tear samples contained IgE antibodies to allergens that were also recognized by serum IgE (eg, patient 23 who mounted strong serum IgE reactivity to rPhl p 1, rPhl p 2, rPhl p 5) contained IgE antibodies to the very same allergens in tears. Tear samples that lacked detectable IgE to certain allergens were frequently from patients who mounted low IgE responses to the very same allergens (patient 5: rPhl p 1, Table II) or contained no IgE antibodies to any of the tested allergens (patient 18: Table II), probably because of dilution during collection of the tear samples.

IgA and IgE antibodies in sera and tears of patients with allergic conjunctivitis detect moieties of different molecular weights in natural pollen extracts

Serum and tear IgE antibodies reacted with components in natural pollen extracts different from those detected by serum and tear IgA (Figs 1, A and B, 2, A and B, 3, A and B, and 4, A and B). The analysis of serum IgA antibodies to nitrocellulose-blotted natural timothy grass and birch pollen extracts showed that allergic as well as nonatopic individuals exhibited IgA reactivity to a great variety of moieties ranging mainly between 20 and 90 kd (Fig 3, A and B). Comparing the pattern of IgA- with that of IgE-defined antigens as well as the intensity of recognition, we noted great discrepancies: sera from grass pollen-allergic individuals displayed IgE reactivity mainly to group 1 and group 5 allergens migrating at approximately 30 kd (Fig 1, A), whereas IgA antibodies of patients and control individuals reacted with moieties of >35 kd (Fig 3, A). Likewise, sera from birch pollen-allergic individuals contained IgE antibodies that were mostly directed against the major birch pollen allergen, Bet v 1, at 17 kd (Fig 1, B), whereas IgA antibodies bound to bands of >30 kd (Fig 3, B). Moreover, tear-derived IgA antibodies of allergic as well as of nonatopic persons reacted with a variety of natural components in birch and timothy grass pollen extract (Fig 4, A and B), which were different from those defined by serum and tear-derived IgE (Figs 1 and 2) or from those defined by serum IgA antibodies (Fig 3, A and B).

Testing with recombinant allergens reveals dissociation of IgE and IgA responses

Comparison of the IgA and IgE antibody specificities with use of defined recombinant pollen allergens documents that serum IgE and IgA reactivity is dissociated (Table II). This is exemplified by the fact that sera that displayed strong IgE reactivity to certain recombinant allergens (eg, sera 16, 17: rPhl p 5; sera 5, 19: rBet v 1) exhibited no IgA reactivity to the very same recombinant allergens (Table II). Furthermore, sera without specific IgE to certain recombinant allergens (eg, sera 8, 9, 11: Bet v 2) contained allergen-specific IgA antibodies to the very same molecules (Table II). Statistical analyses of serum IgE versus serum IgA reactivities to the frequently detected recombinant allergens revealed significant lack of correlation (rPhl p 1: $P = .007$; rPhl p 2: $P < .001$; rPhl

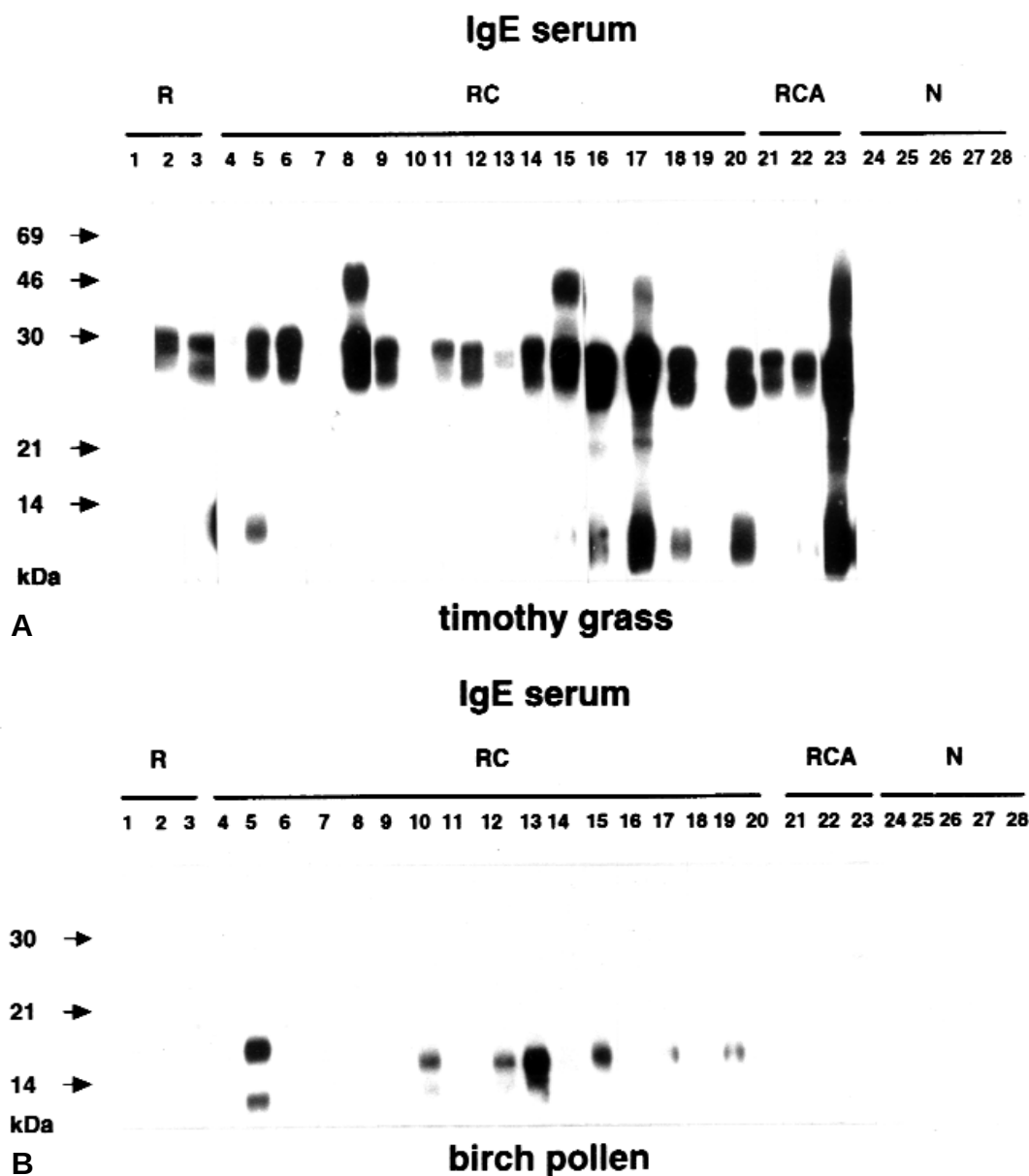


FIG 1. IgE reactivity of sera with nitrocellulose-blotted timothy grass pollen (**A**) and birch pollen extract (**B**). Sera from patients with allergic rhinitis (*R*), allergic rhinoconjunctivitis (*RC*), allergic rhinoconjunctivitis and asthma (*RCA*), and nonatopic (*N*) individuals were exposed to nitrocellulose-blotted pollen extracts. Molecular weights are displayed in kilodaltons on the left side.

p 5: $P < .001$; rBet v 1: $P < .001$), confirming that IgE and IgA responses of allergic patients are dissociated.

IgE antibodies to recombinant allergens could be detected in sera of the allergic patients by ELISA (rPhl p 1: 21/23; rPhl p 2: 18/23; rPhl p 5: 22/23; rBet v 1: 19/23; rBet v 2: 8/23) (Table II). By contrast, no serum or tear sample from the 23 nonatopic individuals contained IgE antibodies against any of the recombinant allergens tested (rPhl p 1, rPhl p 2, rPhl p 5, rBet v 1, rBet v 2) (Table II), which is also expressed by a significant lack of correlation of IgE responses in the aller-

gic versus the nonatopic group (eg, rPhl p 1: $P = .002$; rPhl p 5: $P < .001$).

IgA reactivities to recombinant allergens were found in serum samples of allergic (IgAαrPhl p 1: 15/23; IgAαrPhl p 5: 5/23; IgAαrBet v 2: 4/23) as well as nonatopic individuals (IgAαrPhl p 1: 10/23; IgAαrPhl p 5: 1/23; IgAαrBet v 2: 5/23) (Table II). Statistical analysis confirmed the somewhat weaker lack of correlation of IgA reactivities between allergic versus nonatopic individuals (eg, rPhl p 1: $P = .012$; rPhl p 5: $P = .041$). No tear samples from nonatopic persons and

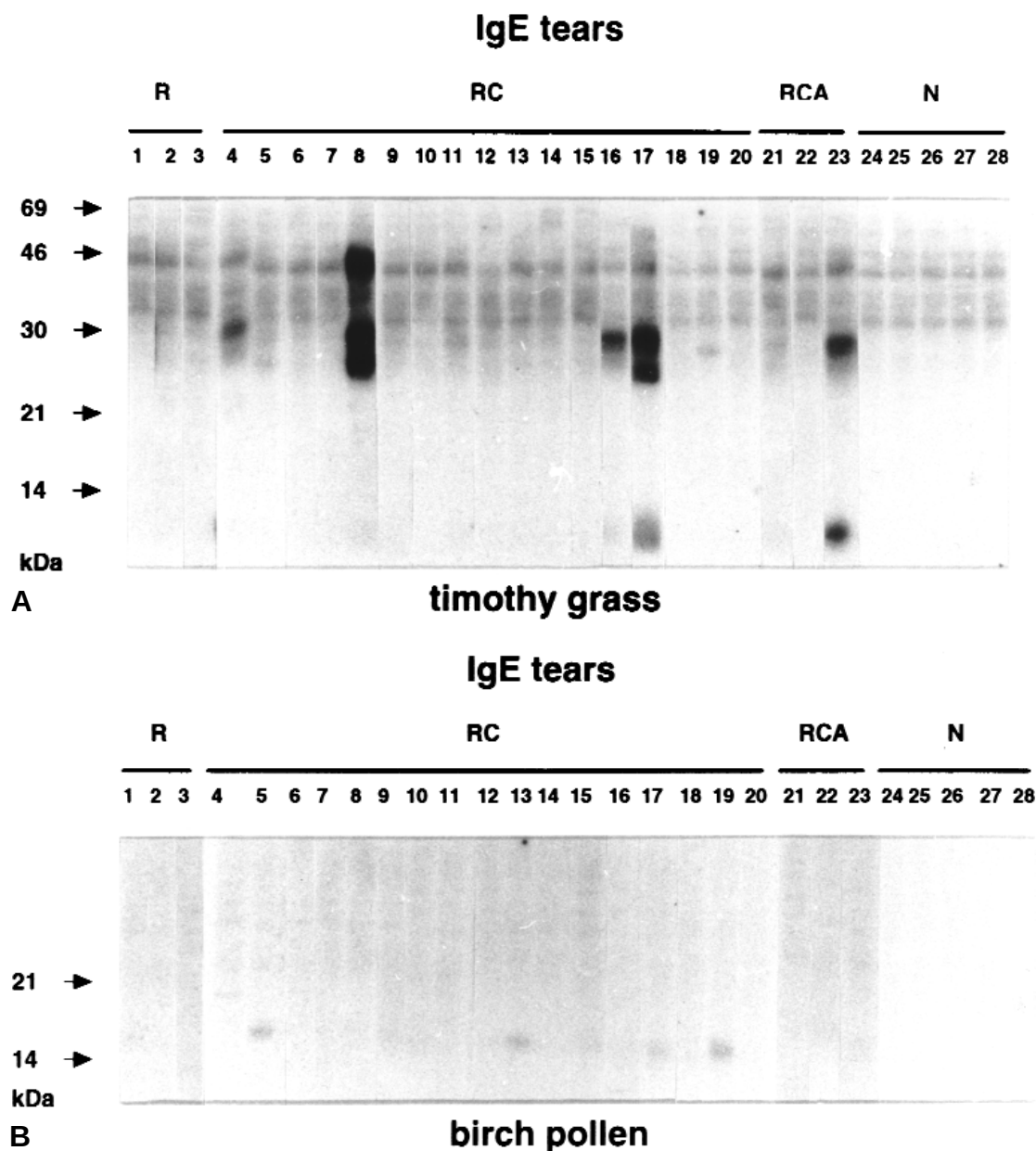


FIG 2. Detection of allergen-specific IgE antibodies in tears. Nitrocellulose-blotted timothy grass (**A**) and birch pollen extract (**B**) were exposed to tears from patients with allergic rhinitis (*R*), allergic rhinoconjunctivitis (*RC*), allergic rhinoconjunctivitis and asthma (*RCA*), and nonatopic (*N*) individuals. Molecular weights are displayed in kilodaltons on the left side.

only 4 tear samples from allergic individuals contained IgA antibodies against recombinant allergens (IgA α rPhl p 1: patients 4, 20; IgA α rPhl p 5: patients 3, 4) (Table II).

DISCUSSION

Seasonal allergic conjunctivitis belongs to the most frequent acute manifestations of mucosal atopy.^{1,33} Although there is evidence for a local production of allergen-specific IgE antibodies in tears of patients with allergic conjunctivitis and for an association of ocular symp-

toms with the amount of allergen-specific IgE in tears,²⁻⁴ little is known regarding the presence of other Ig classes and their specificity in tears. We believe that allergen-specific antibodies other than IgE in local secretions of the eye and respiratory tract may have beneficial effects if they were directed to IgE-defined epitopes. Such local "blocking antibodies" would inhibit IgE recognition of allergens and the subsequent IgE-triggered release of mediators. Because IgA antibodies represent the most prominent class of Igs in mucosal secretions and hence also in tears,³⁴ we were particularly interested to investi-

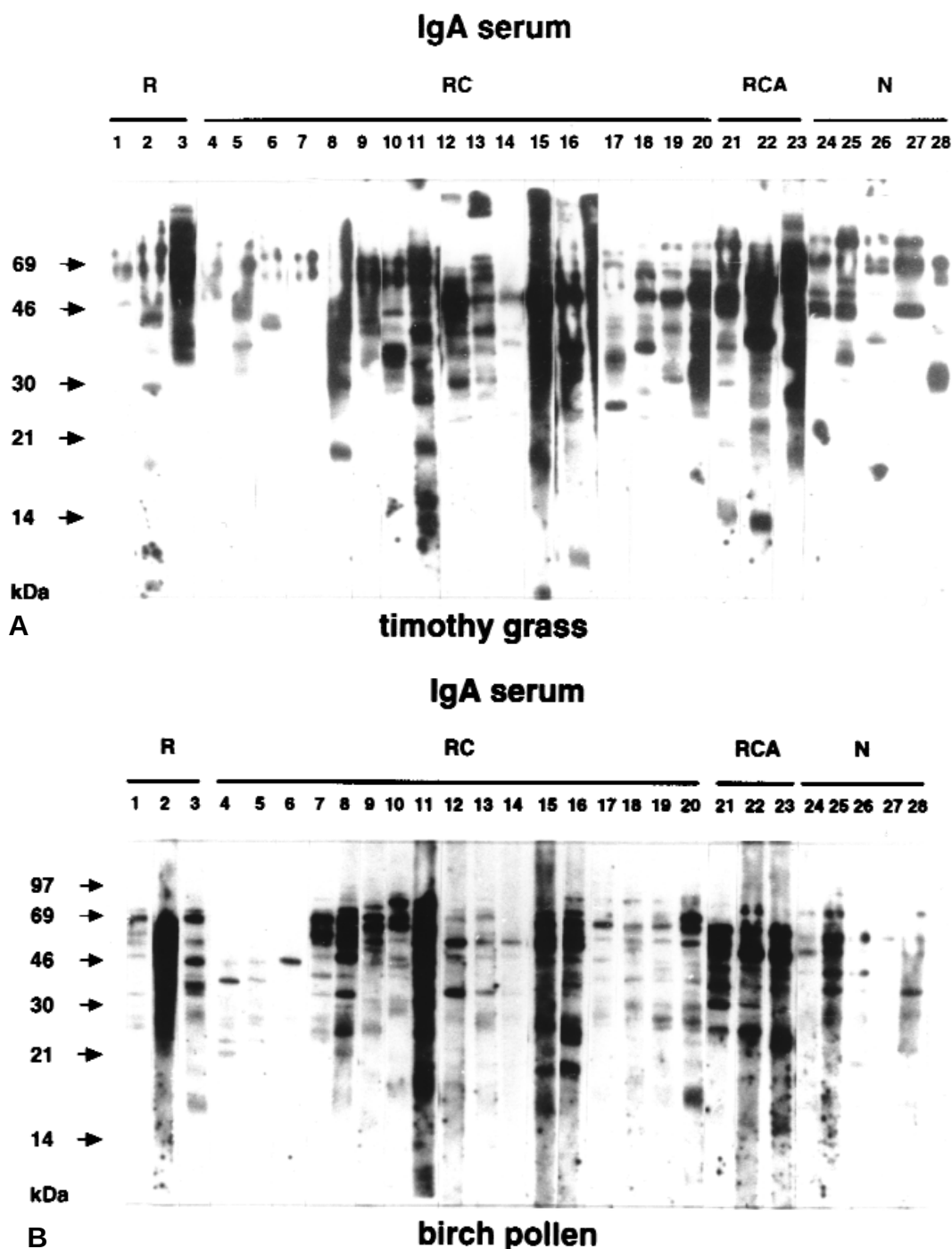


FIG 3. Serum IgA reactivity to nitrocellulose-blotted timothy grass (**A**) and birch pollen extract (**B**). Sera from patients with allergic rhinitis (*R*), allergic rhinoconjunctivitis (*RC*), allergic rhinoconjunctivitis and asthma (*RCA*), and nonatopic (*N*) individuals were incubated with nitrocellulose-blotted pollen extracts. Molecular weights are displayed in kilodaltons on the left side.

gate the possible presence of allergen-specific IgA antibodies in tear samples. To be able to dissect the exact specificities of allergen-specific antibodies in serum and tears, we used purified recombinant timothy grass and birch pollen allergens. The already previously suggested importance of allergen-specific IgE in tears for acute

ocular allergy²⁻⁴ is supported by our finding that allergen-specific IgE antibodies were detected in tears of almost 50% of patients with acute allergic conjunctivitis and that IgE antibodies in tears and serum were mainly directed to the very same allergen components. Although it is well established that patients who predominantly

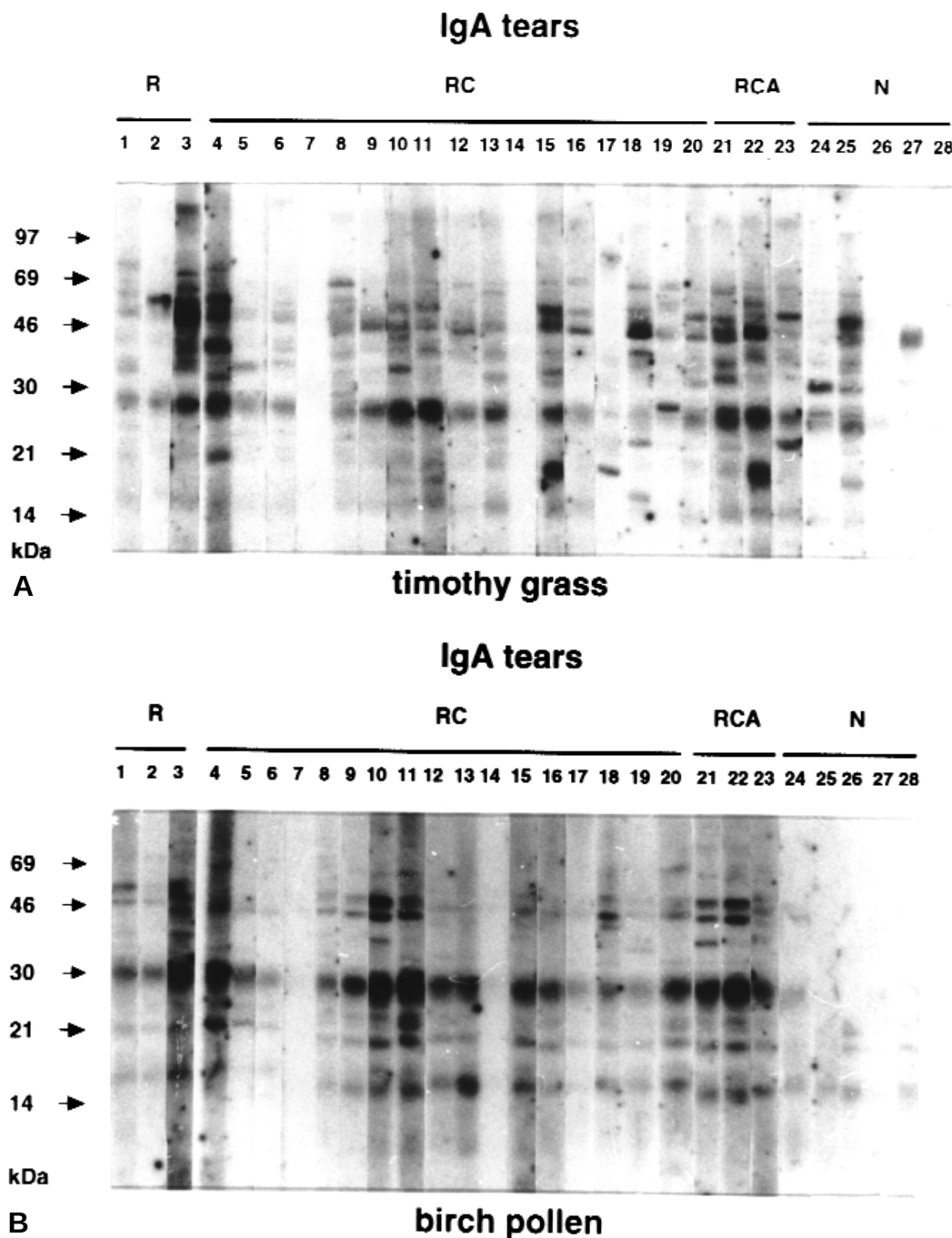


FIG 4. Detection of IgA antibodies in tears with specificity to nitrocellulose-blotted timothy grass pollen (**A**) and birch pollen extract (**B**). Tears from patients with allergic rhinitis (*R*), allergic rhinoconjunctivitis (*RC*), allergic rhinoconjunctivitis and asthma (*RCA*), and nonatopic (*N*) individuals were incubated with blotted pollen extracts and bound IgA antibodies were detected with ¹²⁵I-labeled antihuman IgA antibodies. Molecular weights are displayed in kilodaltons on the left side.

have symptoms of allergic rhinitis can also exhibit ocular symptoms after allergen provocation,³⁵ we found it noteworthy that none of our patients reporting primarily symptoms of allergic rhinitis had allergen-specific IgE in tears. In comparing IgG and IgA antibody responses with IgE responses, we found that the specificities of IgG sub-

class antibodies and IgA were different from those of IgE antibodies. Although serum IgG subclass responses were at least in part directed against timothy grass and birch pollen allergens, the spectrum of serum IgA antibodies was directed mostly against antigenic pollen moieties other than allergens. In comparing serum IgE and IgA

responses to recombinant allergens, we found a statistically significant dissociation that is also exemplified by the finding that several samples contained allergen-specific IgE without IgA or allergen-specific IgA without IgE. Only 4 of 23 tear samples from atopic patients contained allergen-specific IgA antibodies. We believe that this finding deserves attention because it may at least in part explain mucosal symptoms of atopy as being in part due to a lack of protective antibody responses.

The dissociation of IgE and IgA responses may be explained by different regulation of isotype responses. The latter assumption is supported by earlier studies reporting a dissociation of systemic and mucosal humoral immune responses for celiac disease.^{36,37} Our finding that atopic as well as nonatopic individuals were able to mount IgA responses to allergens may be explained by a complex regulation of isotype responses favoring IgA responses. In this context it was reported that transforming growth factor- β knockout mice showed a partial IgA deficiency combined with strong increases in serum IgE levels.³⁸ Attempts to induce blocking allergen-specific IgA antibodies in tears and, perhaps also, in secretions of the respiratory and gastrointestinal tract by vaccination with defined recombinant allergens,¹⁷⁻¹⁹ hypoallergenic allergen derivatives,³⁹ or DNA vaccination⁴⁰ may be considered to prevent or reduce mucosal manifestations of atopy.

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