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CONFLICT OF INTEREST

The authors have no conflicts of interest directly relevant to the contents of this article.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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High-affinity Bet v 1-specific secretory IgA antibodies in nasal fluids protect against birch pollen allergy

To the Editor,

Nasal fluid antibodies act as specific barrier molecules against inhaled antigens through neutralization and shielding mechanisms. They comprise (i) the most abundant and locally produced dimeric secretory IgA (SIgA), (ii) pentameric secretory IgM (SIgM), and (iii) monomeric IgG from passive leakage through blood.¹ The few allergy studies on nasal fluid antibody subclasses showed divergent results regarding quantification of total or allergen-specific reactivities.²⁻⁴ So far, comprehensive profiling of antibody subclasses combined with functionality tests is lacking.

In this study, ten non-allergics (NA) and ten birch pollen allergics (BPA) from Austria were recruited (Ethics Commission Land Salzburg, 01/20/2011). NA were free of allergy symptoms and IgE negative to birch pollen and Bet v 1 (Table 1). BPA suffered from rhinitis/rhino-conjunctivitis, and two patients also presented with asthma. BPA were SPT-positive to birch pollen with medium/high

serum IgE levels to birch pollen (mean = 21.8 kU/L) and Bet v 1 (mean = 23.2 kU/L) (Figure E1).

Using nasal fluids from NA and BPA obtained immediately after the birch pollen season, antibody subclass reactivity to Bet v 1 was determined (Figure 1A). Bet v 1-specific IgE in nasal fluids of BPA was generally low due to the mild sampling technique, but patients with high serum IgE also showed elevated levels in nasal fluids (Table E1). Interestingly, BPA showed significantly higher nasal fluid IgG4 ($p < 0.001$) and IgG ($p < 0.01$) compared to NA. This observation is a consequence of elevated serum IgG4 that accompanies IgE production in allergics, as nasal fluid IgG is not produced locally but originates from serum transudation.^{5,6} Indeed, Bet v 1-specific IgG was also higher in serum of BPAs and correlated well with serum IgG (Table E1). High Bet v 1-specific SIgA and moderate SIgM reactivity was observed, revealing no difference between NA and BPA (Figure 1A).

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TABLE 1 Demographic of study participants and responses to birch pollen

Nasal fluid donor	Age [years]	Sex	Birch pollen		Serum ImmunoCAP		
			Allergy symptoms	Skin prick test	Bet v 1-specific IgE [kU/L]	Birch pollen-specific IgE [kU/L]	Total IgE [kU/L]
NA 1	29	F	None	np	<0.01	0.02	232
NA 2	28	M	None	np	<0.01	<0.01	3.7
NA 3	33	M	None	np	<0.01	<0.01	63.3
NA 4	36	M	None	np	<0.01	<0.01	45.3
NA 5	22	F	None	np	<0.01	0.07	29.8
NA 6	34	M	None	np	<0.01	<0.01	4.6
NA 7	33	F	None	np	<0.01	0.01	89.7
NA 8	40	M	None	np	<0.01	<0.01	31.4
NA 9	32	M	None	np	<0.01	<0.01	5.9
NA 10	29	F	None	np	<0.01	<0.01	46.1
BPA 1	25	M	R	+++	26.7	24.2	133
BPA 2	28	M	RC	++	0.7	1.3	7.3
BPA 3	51	F	RC	++	7.7	9.3	90.4
BPA 4	23	M	R	++++	16.0	19.9	31.4
BPA 5	60	F	RC	++	14.9	15.2	28.3
BPA 6	49	F	RC	++++	6.2	6.0	28.7
BPA 7	22	M	RC	+++	65.3	58.9	200
BPA 8	41	F	RC, asthma	++	4.5	4.0	125
BPA 9	61	F	RC, asthma	++++	12.8	28.5	206
BPA 10	36	F	RC	+++	57.0	64.5	161

Note: NA 1–10, non-allergic nasal fluid donors; BPA 1–10, birch pollen-allergic nasal fluid donors; R, rhinitis; RC, rhino-conjunctivitis; ++, double positive; +++, triple positive; ++++ fourfold positive; np, not performed.

To expand analyses beyond antibody quantification, functionality determined as binding strength of Bet v 1-specific nasal fluid IgG, SIgA and SIgM was analyzed by avidity ELISA.⁵ Whereas IgG and SIgM avidities were similar, SIgA avidities of NA were significantly higher compared to BPA (Figure 1B,C). Avidity indices represent salt concentrations when 50% of bound antibodies are eluted off the protein. This assay allows determination of individual binding strength of antibody subclasses in complex mixtures and is independent of quantities. To cope with diverse immune responses, mucous membranes comprise high- and low-affinity SIgA.¹ We thus conclude that NAs possess more high-affinity Bet v 1-specific SIgA compared to BPAs.

To investigate whether nasal fluid antibodies of NAs and BPAs differ in their capacity to inhibit serum IgE binding to Bet v 1, we conducted a blocking ELISA. This setup mimics allergen capture to prevent Bet v 1 binding to mast-cell bound (mucosal) IgE. Using a serum pool of birch pollen allergics guaranteed a broad IgE repertoire and enabled testing of all nasal fluid antibodies. To relate blocking capacities to antibody isotypes, individual nasal fluids were separated into an SIgA/SIgM-enriched and purified IgG fraction (Figure E2). Complete nasal fluid and the SIgA/SIgM fraction of NA showed significantly higher inhibitory capacities ($p < 0.01$) compared to BPA (Figure 1D). As nasal fluid SIgM levels are negligible,

high-affinity SIgA seems primarily responsible for this blocking effect.

Interestingly, complete nasal fluids of BPA lacked efficient blocking activity despite the fact that purified IgG showed some inhibitory capacity (Figure 1E). Solely in allergics, an interplay of mucosal antibody subclass interaction led to outcompeting of allergen-IgG binding by the high abundance of low-affinity SIgA. This bound SIgA might however in turn elute off during ELISA wash steps or, more likely, high-affinity allergen-specific serum IgE displaces the low-affinity SIgA. As NAs were shown to possess more high-affinity SIgA, such an effect was absent. Here, we first-time demonstrate that healthy individuals exposed to birch pollen mount a distinct nasal fluid antibody repertoire toward Bet v 1. Functional high-affinity SIgA in NA was identified as protective factor that can prevent allergic sensitization through efficient allergen capture in the nasal mucosa. Further studies will reveal the potential of SIgA as diagnostic marker to discriminate allergic and atopic individuals and to monitor the success of allergen-specific immunotherapy.

KEYWORDS

allergens and epitopes, allergy diagnosis, immunologic tests, mucosal immunity, pollen

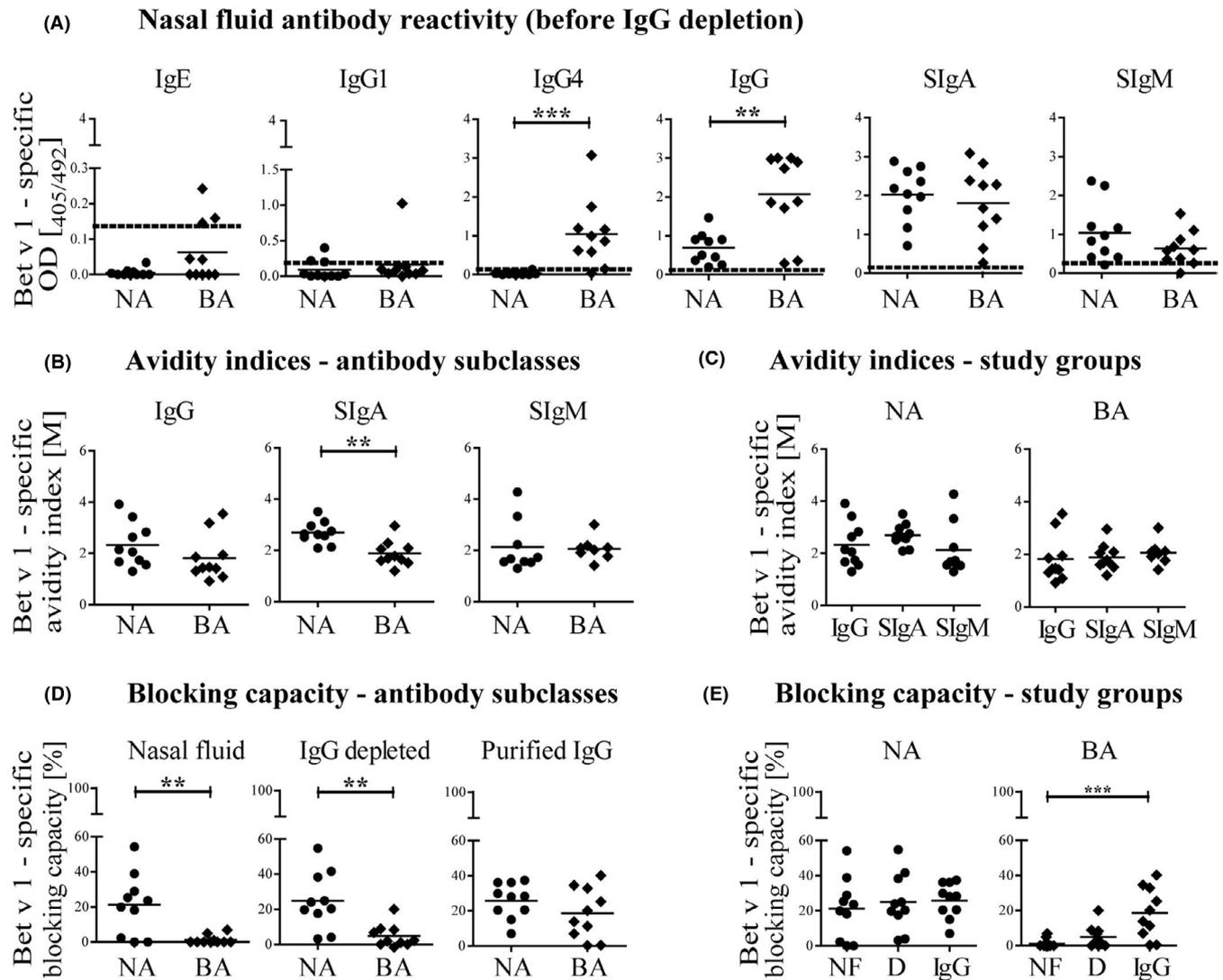


FIGURE 1 Specific antibody reactivity to Bet v 1 in nasal fluids (NFs) of non-allergic (NA) and birch pollen allergic (BPA) individuals. (A) Antibody subclass reactivities in NFs analyzed by ELISA. Mean reactivities are indicated by bars, and dotted lines represent LOD (3xSD of buffer control). Statistics using Mann-Whitney test. (B) Comparison of Bet v 1-specific antibody avidities between study groups. Statistics using Mann-Whitney test. (C) Comparison of mucosal antibody avidities within each study group. Statistics using Kruskal-Wallis and Dunn's post-test. (D) Percentage blocking capacity of NF and fractions to inhibit serum IgE binding to Bet v 1 compared by antibody subclasses. Statistics using Mann-Whitney test. (E) Percentage blocking capacity of complete NF (CNF), SIgA/SIgM enriched NF (SIgA/SIgM) and purified IgG (IgG) to inhibit serum IgE binding to Bet v 1 compared by study groups. Statistics using Kruskal-Wallis and Dunn's post-test. *** $p < 0.001$, ** $p < 0.01$

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CONFLICT OF INTEREST

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Distinct effects of antigen and compound 48/80 in the guinea pig trachea

To the Editor,

Mast cells (MCs) can be activated immunologically by allergen and non-immunologically via the Mas-related G protein-coupled receptor member X2 (MRGPRX2).¹ With the emerging interest of MRGPRX2, we compared the airway smooth muscle (ASM) response and release of mediators in airways exposed to the human MRGPRX2 agonist compound 48/80 (C48/80) and house dust mite (HDM). A guinea pig model was selected for greatest similarity with human airways.²

We measured ASM contraction using organ baths. Both C48/80 and HDM induced marked constriction in trachea from sensitized guinea pigs (Figure 1). The effects of HDM were most likely due to specific IgG which, in addition to IgE, has been shown to induce smooth muscle contractions in guinea pig trachea³ (Figure S1). Antagonizing histamine H₁ receptors inhibited the concentration-response curves and the initial 15 minutes of responses induced by submaximal bolus dose challenges, indicating that the early response of both treatments was mediated by histamine. In contrast, the whole phase responses could only be dampened by inhibition of both histamine and 5-lipoxygenase (5-LOX) pathways, suggesting that both C48/80 and HDM contractions were mediated through histamine and 5-LOX products (Figure 1).

For measuring mediator release induced by C48/80 and HDM, a bolus dose of the strongest concentration of each stimulus was given. Histamine and lipid mediators in the bath fluids were measured by ELISA and a recently developed mass-spectrometry platform with high specificity and sensitivity for detecting lipid mediators.⁴ Both C48/80 and HDM released histamine to similar levels (Figure 2). In addition, 29 lipid mediators were changed after 60 minutes stimulation; 15 were elevated by both C48/80 and HDM challenge, and an additional 13 only by C48/80, while LTE₄ was only elevated by HDM (Figure S2 and Table S1).

The prostanoids were the most abundant bioactive lipid mediators detected in the organ baths after challenges. Prostaglandin (PG) D₂, a major lipid mediator from MCs, together with PGD₁ and PGD₃, were elevated in bath fluids challenged by C48/80 and HDM to similar levels. On the other hand, thromboxane (TX) B₂, was to a greater extent released by C48/80 than HDM, and PGE metabolites, PGF_{2α}, 6-keto-PGF_{1α}, and TXB₃ were solely increased by C48/80 (Figure 2 and Figure S3).

Both C48/80 and HDM increased LTB₄, whereas only HDM triggered the release of cysteinyl leukotrienes (CysLTs) reflected by the elevation of LTE₄ (LTC₄ 0%, LTD₄ 1.7%, and LTE₄ 98.3% of measured products by an additional analysis). In addition to prostanoids and leukotrienes, low levels of other lipid mediators were primarily increased by C48/80 (Figure 2 and Figure S3).

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