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House dust mite-specific IgA2 is associated with protection against eczema in allergic patients

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Short title: IgA2 is associated with less severe HDM allergy

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ABSTRACT

Upon inhalation, house dust mite (HDM) allergens are deposited at the nasal and oral mucosa, where IgA is produced abundantly. IgA subclasses have been linked to protection against respiratory allergy previously. It is currently not known if and how the human IgA subclasses IgA1 and IgA2 contribute to the clinical status of house dust mite-allergic patients. Saliva and serum samples were collected, and HDM-specific, IgE, IgG4, IgA1 and IgA2 levels were determined. HDM-specific levels of IgA in serum were similar to levels measured in non-allergic controls, but HDM-specific levels of IgA2 in saliva were decreased in allergic subjects. HDM-allergic patients that suffered from rhinitis and eczema showed a significant decrease in IgA2-levels compared to patients that in suffered from rhinitis only. Taken together, our findings indicate that HDM-specific IgA2, but not IgA1, levels in serum and saliva are reduced in HDM-allergic patients suffering from eczema.

INTRODUCTION

Levels of allergen-specific IgE in serum often correlate poorly with the intensity of clinical manifestations of allergy(1, 2). Increasing evidence is showing that production of allergen-specific IgG4 antibodies correlate with protection against allergy(3). In addition to IgG4, IgA has been postulated in the past to protect against allergy.

Recently salivary IgA was shown to be associated with protection against allergy(4, 5). IgA is the dominant isotype present at mucosal surfaces, and therefore is the first antibody isotype to come into contact with allergens that are deposited in the airways or the gastro-intestinal tract. The presence of allergen-specific IgA at mucosal surfaces may therefore prevent allergens from entering the body and prevent IgE binding. Humans produce IgA1 and IgA2 which are present at different levels in different mucosal tissues. In line with this, allergen-binding IgA2-, but not IgA1,-producing B cells in nasal tissue have been observed after successful birch-pollen immunotherapy(6).

The aim of this study was to relate allergen-specific levels of IgA1 and IgA2 in serum and saliva to clinical symptoms in house dust mite allergic patients.

MATERIALS AND METHODS

Patients and controls

HDM-allergic patients, (aged 14 years or older), with a positive medical history of allergic rhinitis with or without concomitant clinically stable asthma ($FEV_1 > 80\%$ predicted), and with or without mild to moderate atopic dermatitis (SCORAD < 2) were skin tested with common inhalant allergens (HAL Allergy). Contraindications were severe asthma or use of interfering medication. If applicable, patients had to stop the use anti-histamines three days and steroid nasal sprays one week before the SNPT. Immediately after reaching the provocative nasal threshold, saliva (using salivettes, Sarstedt) and serum samples (serum vacutainers, BD) were collected and stored at -20°C until use. All procedures were performed as specified in the EAACI position article and guidelines(8).The investigations were approved by the local Medical Ethical Committee.

Control subjects did not suffer from allergy. In the inclusion of control subjects (seven males, seven females), no clinical symptoms of allergy or sensitization to house dust mite was allowed. Samples from patients and control subjects were collected outside the pollen season and were negative for HDM-specific IgE (<0.35 kU/L).

Detection of HDM-specific IgA1 and IgA2

HDM extract ($0.2\text{ }\mu\text{g/ml}$, HAL allergy) was coated overnight at 4°C . Plates (Greiner Bio-one) were blocked with biotin free Universal Casein Diluent/Blocker (SDT reagents), and samples diluted 5 and 20 times were incubated in duplicate. Biotinylated mouse anti human IgA1 or IgA2 (Southern Biotech) and streptavidin poly-HRP80 (SDT reagents) was used for detection.

Statistics

Generalized linear models were used for all statistical tests comparing groups (PASW Statistics version 21). For IgE the non-parametric independent samples median test was used.

RESULTS

Patient description

19 HDM-allergic subjects, six males and 13 females, were included in the study and were subjected to the SNPT. Patients with a positive HDM skin test underwent a standardized nasal provocation test (SNPT) to multiple increasing allergen doses as described elsewhere(7). All 19 patients suffered from rhinitis. 10 patients also had eczema, and four of these also suffered from asthma. All 19 HDM-allergic patients had significantly increased serum levels of HDM-specific IgE compared to non HDM-allergic subjects, who did not have detectable levels of HDM-specific IgE.

Nasal provocation of HDM-allergic patients with HDM-allergens mainly caused rhinorrhoea in patients with HDM allergy and concomitant pollen-related hay fever, and symptoms of blocked sinus in patients suffering from HDM-allergy only.

HDM-specific IgA1 and IgA2 levels in serum and saliva

HDM-specific levels of serum IgA1 were similar between HDM-allergic patients and non HDM-allergic control subjects (Figure 1). HDM-specific levels of IgA2 in serum were non-significantly decreased in HDM-allergic patients ($p=0.088$).

As locally produced IgA may be associated with protection against allergy, HDM-specific IgA2 levels were measured in saliva as readily accessible mucosal secretion. Levels of HDM-specific IgA2 in saliva were significantly lower in HDM-allergic patients compared to non HDM-allergic control subjects ($p=0.037$). Similarly, HDM-allergic patients showed a non-significant trend of having decreased levels of HDM-specific IgA1 in saliva (Figure 1).

Progression of allergy is associated with decreased IgA2 levels

Eczema has previously been reported to be inversely associated with IgA levels(9). In our study levels of HDM-specific serum-IgA1, saliva-IgA1 and saliva-IgA2 were not different between HDM-allergic patients with or without eczema (Figure 1). However, HDM-specific levels of IgA2 in serum were significantly lower when

HDM-patients also suffered from eczema ($p=0.004$). Four of the ten HDM-allergic patients that suffered from eczema also suffered from asthma. Therefore we also analysed the relation between HDM-specific IgA2 and the combination of eczema and asthma. Levels of HDM-specific IgA2 were further decreased in patients suffering from eczema and asthma compared to patients suffering from eczema alone ($p=0.063$) and HDM-allergic patients not suffering from eczema or asthma ($p<0.001$, Figure 2A).

To test the hypothesis that mucosal allergen-specific levels of IgA2 may prevent binding of HDM allergens to IgE, we correlated the ratio between specific saliva IgA2 and IgE with the PNIF and the maximally tolerated HDM dose (in SNPT). The ratio of HDM-specific IgE over saliva IgA2 correlated significantly ($p=0.033$) with acute clinical response to allergen challenge (Figure 2B), but this was not seen for serum IgA2 (not shown). This indicates that mucosal specific IgA2 protects against allergic responses following allergen challenge.

DISCUSSION

The aim of this study was to identify how IgA subclasses relate to the clinical status of house dust mite-allergic patients. HDM-allergic patients had significantly lower levels of HDM-specific IgA2 in saliva, and in serum levels of specific IgA2 were significantly lower in patients with eczema and/or asthma. The ratio of allergen-specific serum-IgE over saliva-IgA2 was associated with the clinical status of the patients. The data reported here demonstrate that the location and subclass of IgA needs to be taken into account to establish associations between allergen-specific IgA and HDM allergy.

The fact that not specific IgA2 levels alone but rather the ratio of serum-IgE over salivary-IgA2 correlated significantly with clinical symptoms in the nose suggests the protective capacity of mucosal IgA2 depends on the IgE levels. In line with our hypothesis, local levels of allergen-specific IgA2, but not systemic serum levels of IgA2 correlated with nasal symptoms upon allergen challenge. In another study, increased levels of salivary, but not serum, IgA after receiving sublingual peanut immunotherapy was associated with higher peanut consumption(10). In contrast to serum IgA, IgA from mucosal secretions may inhibit histamine release, as shown in patients receiving ragweed-immunotherapy(11). Thus, allergen-specific IgA may block entry of allergens at mucosal sites(6).

The presence of eczema correlated with reduced levels of systemic (serum) HDM-specific IgA2, but not serum-IgA1 or mucosal IgA1 and IgA2 (saliva). It should be noted that eczema reported in our patients is not

necessarily caused by house dust mite allergy, for it is difficult to identify the exact cause of this type of eczema. It is unclear how systemic IgA2 may protect against eczema, while this association was not found for HDM-specific serum-IgA1.

To conclude, allergen-specific IgA2 levels in serum (skin) and saliva (nose) are associated with reduced symptoms of allergy. As local production of IgA2 probably occurs under the influence of tissue factors, future work should focus on mechanisms to specifically increase allergen-specific IgA2 levels in serum and mucosal sites to protect against development of asthma and eczema.

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LEGENDS TO FIGURES:

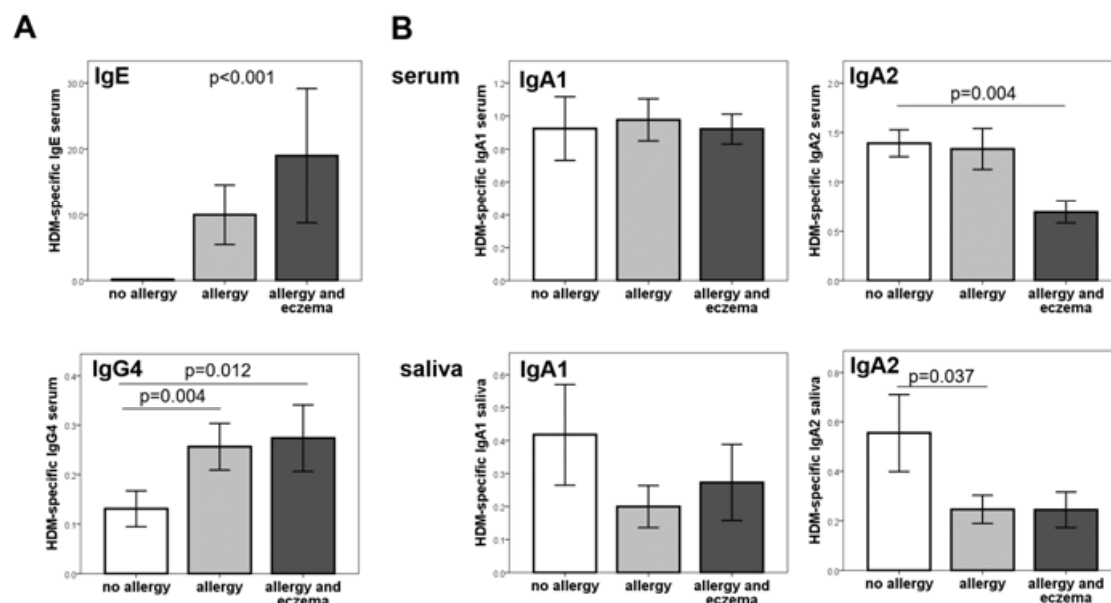
Figure 1. (A) HDM-specific levels of IgE and IgG4 of non-allergic controls and patients with rhinitis alone or also eczema. (B) HDM-specific levels of IgA1 (left) and IgA2 (right) in serum (top) and saliva (bottom) of HDM-allergic and non HDM-allergic individuals with or without eczema. Error bars indicate standard error, and p-values of significant differences are indicated.

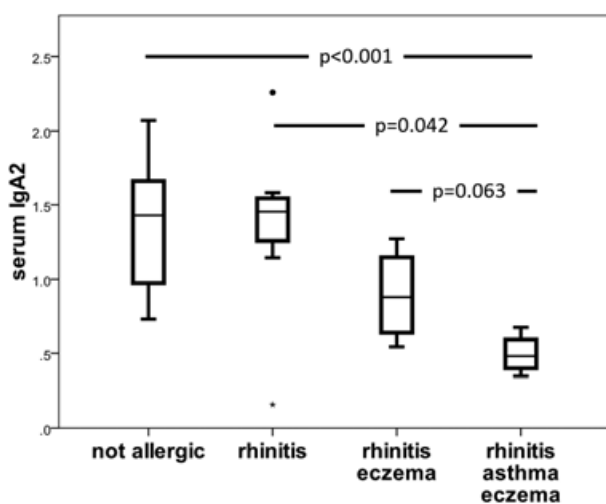
Figure 2 (A). Increased symptoms of HDM-allergic patients are associated with decreased levels of HDM-specific IgA2 in serum, as determined by absence or presence of eczema and asthma. Significant differences are indicated by p-values. Box plots indicate median (horizontal bar), 50% data range (box) and 1st and 4th quartile range (error bars), including all data points, except for a single outlier (○ and *, are outliers). (B) PNIF and maximum tolerated dose during intra-nasal challenge with incremental doses of HDM allergens were combined in a single score and correlated with the ratio of specific mucosal IgA2 over specific serum-IgE.

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