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Dramatically decreased T cell responses but persistent IgE upon reduced pollen exposure

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Abstract

Mugwort pollen allergy is frequent in parts of Europe. As mugwort pollen contains only one major allergen, Art v 1, which harbors only one T cell epitope, we employed mugwort pollen allergy as a model to study allergen-specific T cell responses. However, after 2004, we noticed a drastic decrease in the T cell responses to Art v 1 and eventually it became almost impossible to detect allergen-specific responses at the T cell level in mugwort-allergic individuals. To explain this observation, we retrospectively investigated the local exposure to mugwort pollen and its possible correlation to the frequency and reactivity of allergen-specific T cells. The total annual pollen indices dramatically dropped after 2004 and never reached previous levels again. Local sensitization to mugwort pollen and serum IgE antibodies specific for Art v 1 remained unchanged until 2015. Our mugwort pollen model shows that specific IgE-levels are maintained for extremely long time periods in spite of a long-term reduction of natural allergen exposure to levels that are too low to boost specific T cells.

Keywords

Allergy; Allergen-specific T cells; IgE; Pollen exposure; Sensitization

1 Introduction

Allergen-specific CD4+ T cells play a major role in IgE-mediated allergy. Studies on their contribution to the pathophysiology and cure of allergy have relied on cells obtained from

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.imbio.2019.07.003.

Declaration of Competing Interest

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peripheral blood. However, the frequency of circulating allergen-specific CD4+ T cells is very low. For seasonal allergens 0.001-0.05% peptide-specific T cells as identified by human leukocyte antigen (HLA) class II/peptide tetramers have been reported, however, natural exposure during the pollen season can boost frequencies up to 1000-fold (Wambre et al., 2011, 2012; van Overtvelt et al., 2008). IgE-mediated allergy to mugwort (*Artemisia vulgaris*) pollen is the 3rd most frequent pollen allergy in large parts of Europe (Heinzerling et al., 2005). We began to study mugwort pollen-specific T cells in 1999. Because of scarcity, allergen-specific CD4+ T cells were expanded from PBMC of mugwort pollen-allergic individuals by stimulation with Art v 1, the single major allergen in mugwort pollen (Himly et al., 2003; Jahn-Schmid et al., 2002, 2005, 2008). These Art v 1-specific T cell lines (TCL) and T cell clones (TCC) allowed a detailed characterization of T cell epitopes, their interaction with HLA class II molecules and allergen-specific T cell receptors. Art v 1 became of special interest because it contains one single, immunodominant, HLA-DR1-restricted T cell epitope, Art v $1_{25\text{-}36}$ (Himly et al., 2003; Jahn-Schmid et al., 2002, 2005, 2008). When HLA class II/peptide tetramers became available, we applied HLA-DR1/Art v $1_{25\text{-}36}$ tetramers to unequivocally identify allergen-specific T cells *ex vivo* (Van Hemelen et al., 2015). During the last decade we observed a markedly diminished T cell response to Art v 1, despite the fact that blood sampling was always done within or shortly after the pollen season, using the same parameters for patient selection and in vitro culture systems validated by negative and positive control cultures.

In Poland, where mugwort is very common, mugwort pollen indices recently have dropped markedly, presumably due to climate changes with higher temperatures and draught in early summer (Bogawski et al., 2014). In Vienna, similar climate changes and less open areas due to increased construction activity may have limited growth and pollen release of mugwort plants. We hypothesized that a reduced exposure to mugwort pollen was the cause of the decreasing T cell reactivity in our area. Therefore, we analyzed in retrospect the frequency and proliferation of peripheral Art v 1-specific T cells in mugwort-allergic subjects and compared it to the pollen counts and the sensitization and allergen-specific IgE levels of the local population over a long time period to determine indirectly whether the reduction of allergen-specific CD4+ T cells has an impact on IgE-levels of allergic individuals.

2 Methods

2.1 Study population

Peripheral blood from 116 subjects living in the Vienna area was taken between August and November during the period of 1999-2010. The diagnosis of mugwort pollen allergy was based on typical case history, positive skin-prick-tests and specific serum IgE ≥ 0.35 kU/L to mugwort pollen extract as measured by ImmunoCAP. The individuals who donated blood for T cell experiments had mugwort pollen-specific IgE ≥ 3.5 kU/L (ImmunoCAP class 3). At the time of blood donation, none of the subjects took immunosuppressive drugs or had undergone allergen-specific immunotherapy. Art v 1-reactivity of serum IgE was confirmed by enzyme linked immuno sorbent assay (ELISA) and IgE immunoblots as described (Jahn-Schmid et al., 2002). The study had been approved by the local ethics committee (EK497/2005) and all participants gave informed consent.

2.2 T cell responses

Peripheral blood mononuclear cells (PBMC) were isolated from heparinized peripheral blood to obtain Art v 1-specific TCL and TCC as described (Jahn-Schmid et al., 2002). Briefly, PBMC were stimulated with Art v 1 for 3 days and then expanded with suboptimal concentrations of IL-2 and irradiated PBMC for 21 days. TCC were obtained by limiting dilution of TCL at day 9 of cell culture. TCC were screened with Art v 1 and overlapping synthetic 12-mer peptides spanning the amino acid sequence of Art v 1. Proliferation was measured by thymidine-incorporation as described (Jahn-Schmid et al., 2002) and expressed as stimulation indices (SI), i.e. the ratio of T cells stimulated with allergen/peptide to T cells in medium). As internal positive control, T cells were stimulated in parallel with 20 U/ml IL-2 and 0.5% phytohemagglutinin. Stainings with Human leucocyte antigen (HLA)-DRB(beta)1*01:01/Art v $1_{19\text{-}36}$ tetramer or HLA-DRB1*01:01/CLIP (class II-associated invariant chain peptide) control tetramers (Beckmann Coulter, Marseille, France) were performed as described (Van Hemelen et al., 2015).

2.3 IgE sensitization

The long-time trend in the local prevalence of sensitization to mugwort pollen was calculated based on data from Hemmer et al. (2010). Briefly, skin prick tests using a panel of 16 locally important inhalant allergens including mugwort pollen extract (ALK-Abello, Copenhagen, Denmark) were routinely performed at the allergy clinic Floridsdorf (FAZ) in Vienna in 13,719 consecutive subjects with suspected respiratory allergy between 1997 and 2007. The incidence of mugwort pollen sensitization within the atopic population was evaluated. After the ImmunoCAP to measure IgE specific for natural Art v 1 (w231, Thermo Fisher Scientific) became available, from 2010 to 2015 sera from all individuals with positive skin prick test to mugwort pollen extract (n = 8966) were tested.

2.4 Pollen counts

Airborne pollen concentrations were evaluated with a volumetric pollen and spore trap of Hirst design (Hirst, 1952). Evaluation procedures followed the recommendation guidelines of the European Aerobiology community. Daily mugwort pollen concentrations were summed up each year and total annual pollen counts are shown (Galán et al., 2014).

2.5 Statistics

For statistical analyses the Mann-Whitney-U test or the Kruskal-Wallis plus Dunn’s multiple comparison test were performed using GraphPad Prism 5 (GraphPad Software Inc.) Differences were considered statistically significant for values of $p \leq 0.05$.

3 Results and discussion

In the period from 1999 to 2013 we expanded Art v 1-specific T cell lines (TCL) from mugwort pollen allergic donors which reacted to the immunodominant peptide Art v $1_{25\text{-}36}$ with median stimulation indices (SI) ranging from of 3.6 to 10.2. After 2004, the T cell responses decreased to median SI of 1.7-4.3. (Fig. 1A). This reduced T cell reactivity was also reflected in the lower number of Art v 1-specific T cell clones (TCC) that could be established from Art v 1-induced T cell lines during 1999–2003 compared to 2005–2008

(Fig. 1B; Mann-Whitney-U Test; p = 0.0156). Fig. 1C demonstrates the decreased yield of Art v 1-specific TCC in 2006 and 2007 as compared to time points before 2004 from the two individuals who donated blood more than once. After 2007, we could not establish Art v 1-specific TCC although the cloning efficacy itself did not change during this time. In 2005 we started to use HLA-DR1/Art v 1-peptide tetramers to identify Art v $1_{25\text{-}36}$-specific CD4+ T-cells in TCL from mugwort-allergic individuals. In 2005 we detected a maximum of 15.9% tetramer + CD4+ T cells, and in 2006 a maximum of 6.3% in TCL raised under the same conditions. The median value drastically dropped from 6.9% to 0.8% tetramer + CD4+ T cells ($p < 0.01$; Fig. 1D).

We assumed that the reduction of allergen-specific T cells in peripheral blood was due to insufficient boosting. Therefore, we analyzed the local mugwort pollen exposure in Vienna from 1997 to 2018. Until 2004 the annual pollen indices were relatively constant and amounted to 720 ± 208 (mean ± SD; not shown). A transient drop of the annual pollen counts below 600 was recorded in 1999 and 2000. In 2005 another prominent drop was observed. Thereafter, annual pollen indices never reached previous levels again, amounting to 362 ± 118 for the period of 2005-2018. As shown in Fig. 2A, the total annual pollen counts drastically dropped from around 800 in 2003 and 2004 to values around 400–500 pollen in the following years and further decreased to levels of 257 ± 66 since 2013 (Fig. 2A).

To find out whether this decrease in pollen exposure also affected the IgE-response of mugwort pollen in allergic individuals we went back to a study that had investigated the sensitization to mugwort and ragweed pollen in Eastern Austria. Skin prick tests with mugwort pollen extract that had routinely been performed to 13,719 subjects during the years 1997–2007 were analyzed bi-annually (Hemmer et al., 2010). The incidences of positive skin prick tests steadily increased from 1997 to 2007 (n = 1126–1887) (Fig. 2B). Thus, the sensitization rates were not affected by these decreased pollen counts since 2005. From 2009, ImmunoCAP assays for the marker allergen Art v 1 have been implemented for unequivocal in vitro diagnosis of mugwort pollen sensitization and exclusion of any kind of cross-reactivity. The percentage of Art v 1-positive sera (≥ 0.35 kU/L IgE) in all subjects with positive skin prick test for mugwort extract largely remained unchanged between 2009 and 2015, varying between 38.6% and 33.3% and reaching a maximum of 40.1% in 2013 (data not shown). In addition, the percentage of Art v 1-positive subjects within the different CAP-classes was calculated. However, no significant changes were observed (Fig. 2C). An intra-individual comparison of Art v 1-specific IgE-levels in different years was only possible in one subject showing a slight decrease in post-seasonal Art v 1-specific IgE (Fig. 2D). These data demonstrate that, although the pollen exposure decreased markedly within the last decade, the prevalence of sensitization and IgE-levels did not change.

To the best of our knowledge this is the first human study examining the effects of naturally reduced exposure to inhalant allergens on both, T cell responses and IgE-levels. In our retrospective study, a marked drop of mugwort pollen exposure since 2005 (Fig. 2A) most likely explains the significant decrease in peripheral Art v 1-specific T cells of mugwort-allergic subjects. In 2006, after the first 2 years of low exposure, reactive T cells were already significantly reduced and disappeared totally thereafter, indicating that the remaining

pollen concentration was insufficient to maintain circulating Art v 1-specific T cells. In contrast, sensitization and Art v 1-specific IgE-levels remained constant for several years (Fig. 2B-D) despite decreased allergen exposure. To achieve a decrease in specific IgE total avoidance of allergen seems to be required, as it has been reported that an environment free of house dust mites led to significantly reduced allergen-specific IgE-levels after 3 and 9 months (Sensi et al., 1994), whereas reduced exposure to cats for 8 months left IgE-levels unchanged (Erwin et al., 2014). Our mugwort model shows maintenance of specific IgE-levels for extremely long time periods in spite of allergen exposure levels which are too low to boost specific T cells in numbers detectable in the peripheral blood. In mice, it has been demonstrated that IgE can be produced T cell-independently by long-lived plasma cells for long time periods (Luger et al., 2009). Moreover, T cell-independent plasma cell differentiation from pre-existing memory B cells and IgE production has been reported (Lutz et al., 2017). In humans, there are no data available that would directly explain the long-lasting production of IgE in the absence of T cell help. Indirectly, persistence of IgE responses have been observed in AIDS patients with very low CD4 T cell counts (Marth et al., 2014) and in long-term cyclosporin A treated subjects (Lucae et al., 2015). On the other hand, the presence of non-circulating, allergen-specific, resident CD4+ T cells in mucosal tissue was demonstrated in a mouse model of asthma. (Hondowicz et al., 2016), and local IgE-production has been observed in human nasal tissue *ex vivo* (Cameron et al., 2006; Takhar et al., 2005). However, whether such local IgE-production can be the source of relevant amounts of allergen-specific serum IgE is debatable.

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Abbreviations

PBMC	peripheral blood mononuclear cells
SI	stimulation index
TCC	T cell clones
TCL	T cell lines

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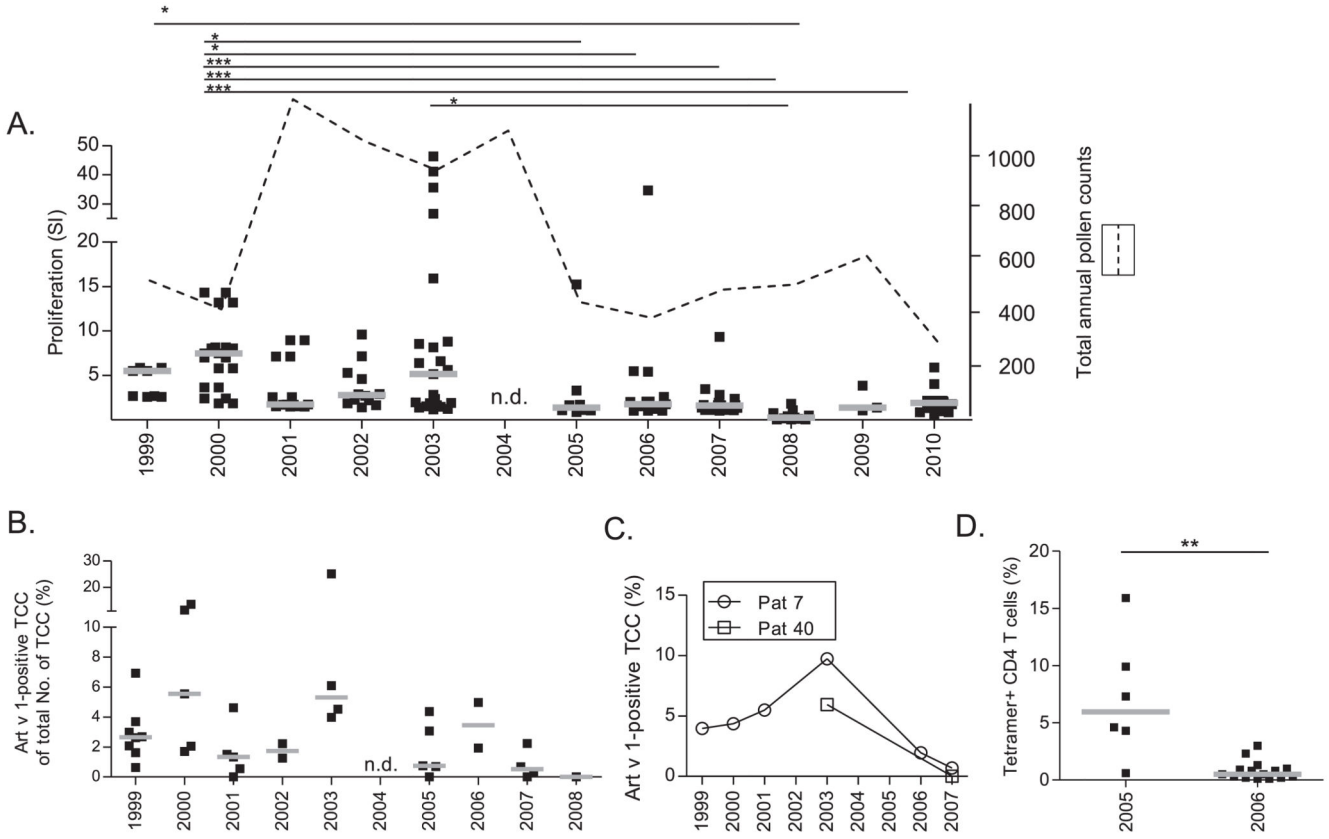

Fig. 1. Decreased pollen exposure and T cell responses to Art v 1 in Vienna after 2004.

(A.) T cell proliferation in response to Art v $1_{25\text{-}36}$ in Art v 1-induced T cell lines (TCL) derived from PBMC of mugwort pollen allergic individuals is shown. Blood was taken in Vienna during the same season each year from 1999-2010. (*p < 0.05, ***p < 0.001; Kruskal-Wallis and Dunn's multiple comparison post hoc test). The dashed line shows annual total pollen counts in Vienna during that time period. (B.) T cell clones (TCC) were established from Art v $1_{25\text{-}36}$ -specific TCL derived from different subjects. The percentage of Art v $1_{25\text{-}36}$ -specific TCC in total number of TCC obtained in the cloning experiment from one donor is shown. (C.) The two individuals who donated blood recurrently are shown separately (Pat 1/Pat 2). (D.) Percentage of HLA-DR1/Art v $1_{19\text{-}36}$ tetramer-positive cells in Art v 1-induced TCL obtained from 8 and 19 different donors, respectively. (**p < 0.01; Mann-Whitney U test).

n.d., not done - unfortunately in 2004 our lab concentrated only on ragweed sensitized individuals; SI, Stimulation index (SI): ratio of cpm in TCL stimulated with Art v 1-peptide and TCL with medium control in the presence of irradiated antigen presenting cells.

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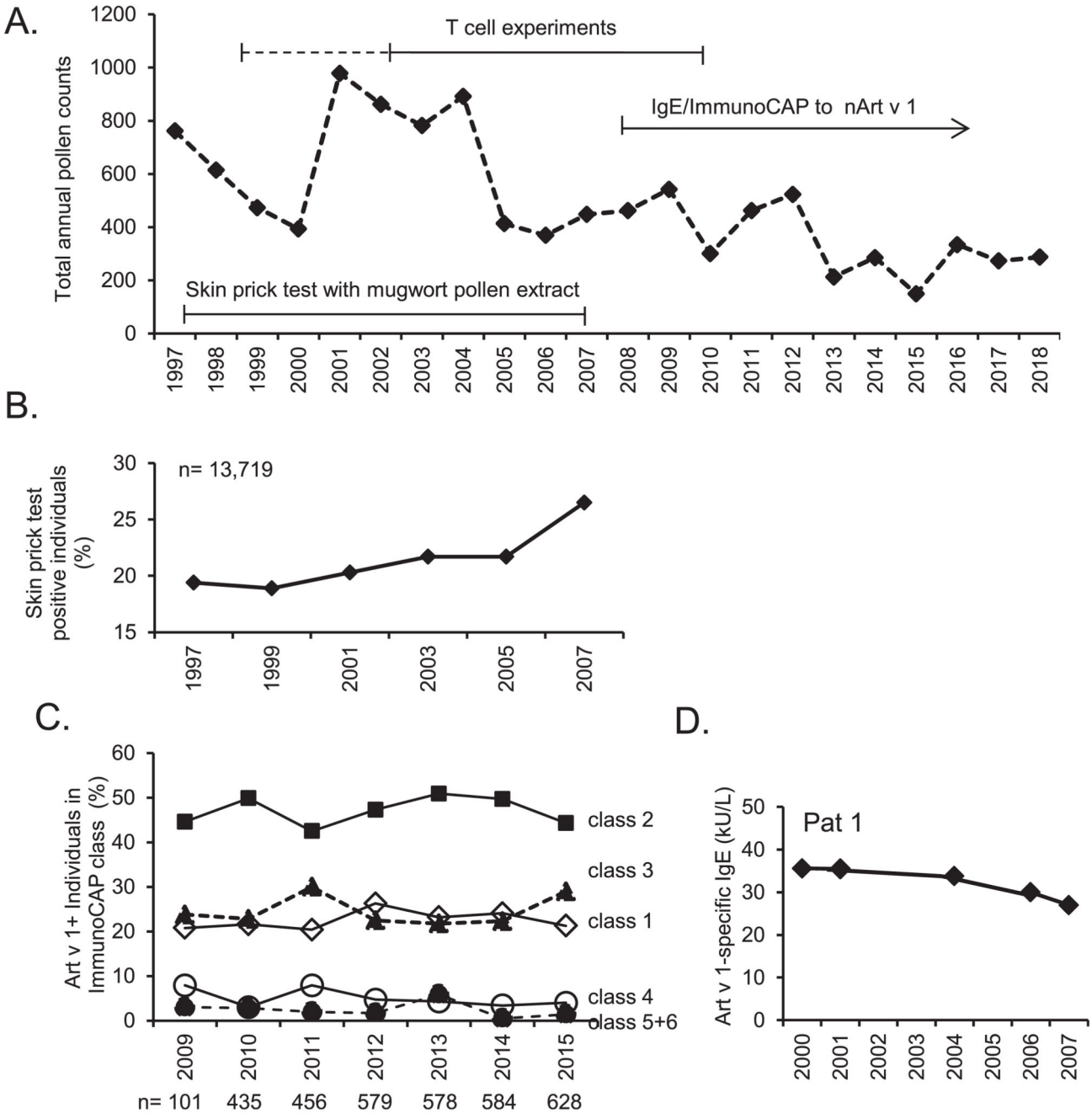

Fig. 2. Exposure and prevalence of sensitization to mugwort pollen in Vienna from 1997-2018.

(A.) Local total pollen counts for *Artemisia vulgaris*. The years when T cell experiments were performed and when sensitization of the population was assessed are indicated. (B.) Percentage of mugwort pollen-sensitized subjects as determined by skin prick test with pollen extract (1997–2007) and (C.) analysis of Art v 1-specific serum IgE-levels within all mugwort positive subjects (2009–2015) and (D.) within a single individual (2000–2007)

(Pat7 is identical to Pat7 in Fig. 1C). ImmunoCAP classes are defined as follows (kU/L):1, 0.35–0.70; 2, 0.7 0–3.5; 3, 3.5–17.5; 4, 17.5–50; 5, 50–100; 6, > 100.

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