CONFLICT OF INTEREST

The authors identify no conflict of interest.

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SUPPORTING INFORMATION

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

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Chemical modification of ragweed extract results in an increased safety profile while maintaining immunogenicity

To the Editor,

Short or common ragweed (*Ambrosia artemisiifolia*), belonging to the plant family of Asteraceae, is an annual weed with pollination peak levels in late summer. Epidemiological studies revealed a sensitization prevalence of 23%–32.8% for the US population, whereas in European countries the prevalence shows more variety, for example, 3.5% in Italy and 54% in Hungary.[1] Due to the rapid dispersal of ragweed, its massive pollen production, and high allergenic potential, ragweed allergy is developing into a significant global health concern. To date, 11 allergens have been recorded in the official IUIS allergen database, with Amb a 1 representing the most clinically relevant allergen with sensitization rates >90%.[2,3]

Abbreviations: AIT, allergen-specific immunotherapy; $Al(OH)_3$, aluminum hydroxide; FAB, IgE-facilitated allergen binding; IUIS, International Union of Immunological Societies; MRE, modified ragweed extract; MS, mass spectrometry; RE, ragweed extract; SCIT, subcutaneous immunotherapy; SLIT, sublingual immunotherapy.

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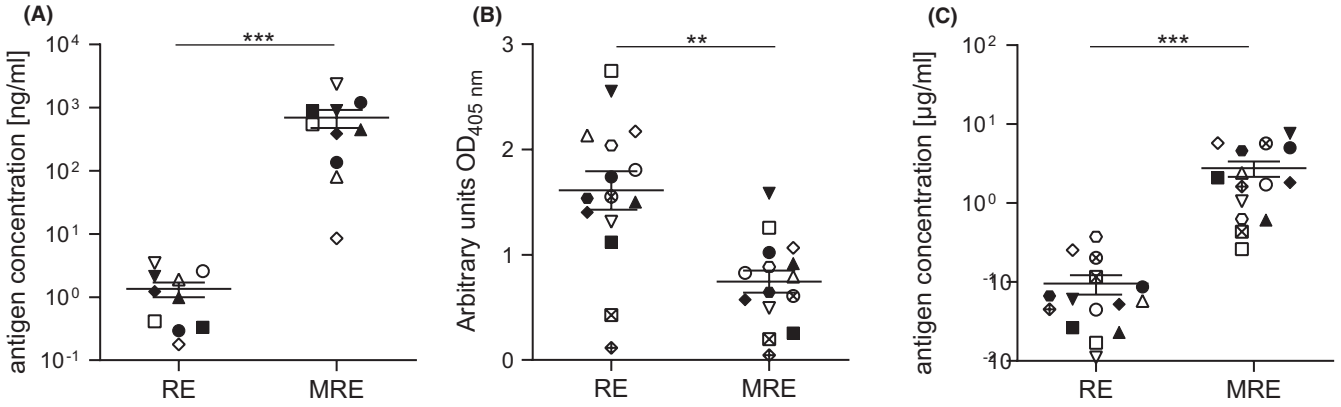

FIGURE 1 Mediator release assay (huRBL). A, Mediator release assay (huRBL). Antigen concentration needed to reach 25% ß-hexosaminidase release is 500-fold higher in MRE compared with RE (patients 14 and 15 were excluded from analysis since stimulation with MRE did not reach the 25% of the ß-hexosaminidase release). B, IgE-binding capacities of RE and MRE were analyzed by indirect ELISA (n = 15). C) IgE cross-reactivity between RE and MRE was addressed by inhibition ELISA experiments (n = 15). Statistics were calculated by the Mann-Whitney test and unpaired t test. $^{**}p < 0.01$ and $^{***}p < 0.001$

At present, allergen-specific immunotherapy (AIT) represents the only treatment for respiratory allergies leading to long-lasting clinical benefits up to permanent immune tolerance after treatment discontinuation. Subcutaneous immunotherapy (SCIT) generally shows more extensive effects compared with sublingual immunotherapy (SLIT)[4]; however, at present no commercially SCIT is available for ragweed. Therefore, a subcutaneous chemically modified ragweed-based immunotherapy product (MRE) is being developed. In the present study, the immunogenicity and allergenicity of MRE in comparison with a common ragweed extract (RE) were evaluated.

Modified ragweed-based immunotherapy product was generated by cross-linking of standardized ragweed pollen extract with glutaraldehyde followed by adsorption to aluminum hydroxide ($Al(OH)_3$). Mass spectrometry (MS) analyses of MRE verified the conservation of all major allergens and minor allergens with the exception of Amb a 9 and Amb a 10. Furthermore, various peptides of the five known Amb a 1 isoforms could be clearly identified (Table S1). Dynamic light scattering (DLS) analyses showed that MRE has a hydrodynamic radius of 10.78 nm. This radius and the type of peak formation indicate a single aggregated population, verifying that chemical modification was successful (Figure S1A).[5,6]

Allergenicity of MRE vs. RE was assessed by mediator release experiments using ragweed-allergic patient sera. Sera were obtained from 15 subjects (8 males and 7 females, mean age: 35.5) with clinical history of ragweed pollen allergy, positive skin prick tests, and comparable total IgE ImmunoCAP values. Immunoblot analysis using RE confirmed IgE reactivity toward ragweed allergens (Figure S2). Experiments using anonymized serum samples of ragweed-allergic patients from Austria were approved by the Ethics Committee of the Medical University of Vienna (No. 712/2010), and informed written consents were obtained. Subjects' clinical and serologic characteristics are summarized in Table S2. Mediator release assays were performed using RBL-2H3 cells carrying the human FcεRI alpha chain.[7] RBL-2H3 cells were passively sensitized with human sera. Serial dilutions of RE or MRE were used to trigger IgE receptor cross-linking followed by allergen-dependent ß-hexosaminidase release from cells into the supernatant. Results revealed that RE induces higher overall mediator release than MRE in ragweed-allergic patients. For patients 14 and 15 (both Amb a 1 non-responders; Figure S2), very limited activation with MRE was observed (Figure S3). Calculations of the antigen concentration at which 25% ß-hexosaminidase release was reached showed that statistically significantly more MRE (mean of 696.96 ng/ml) was needed to induce the same amount of mediator release as RE (mean of 1.35 ng/ml) (Figure 1A). Additionally, IgE-binding capacities of RE and MRE were tested by indirect ELISA using 15 ragweed-allergic patients' sera showing a statistically significant reduction in IgE binding against MRE (Figure 1B). These results suggest a significantly lower allergenic potential of MRE compared with RE, which was further verified by inhibition ELISA using concentration series of RE or MRE, respectively, to inhibit IgE binding to RE (Figure 1C).

Immunogenic properties of MRE were evaluated in an in vivo mouse model by measuring induction of RE- or Amb a 1–specific IgG1, IgG2a, IgE, and total IgE levels. Animal experiments were performed according to the guidelines of the Austrian Federal Ministry of Science, Research, and Economy (BMWF-66.012/0017-WF/V/3b/2017). After 4 immunizations with formulated RE and MRE, RE-specific and natural Amb a 1–specific IgG1 levels (Figure 2A,B) were increased for MRE compared with RE. IgG2a was only slightly induced in all treated groups with no significant difference (data not shown). Moreover, total and specific IgE levels (Figure S5A,B,C) were lower in MRE-immunized mice than RE-immunized mice, suggesting a lower risk for allergic side effects during SCIT. These results indicate that MRE not only induces less total and specific IgE compared with RE but is also a stronger inducer of IgG1 antibodies, which are cross-reactive with unmodified ragweed pollen proteins.

The allergenic potency of IgE induced by MRE immunizations in comparison with RE immunizations was addressed by murine mediator release experiments. Results showed that immunizations with RE induced slightly higher ß-hexosaminidase release in comparison

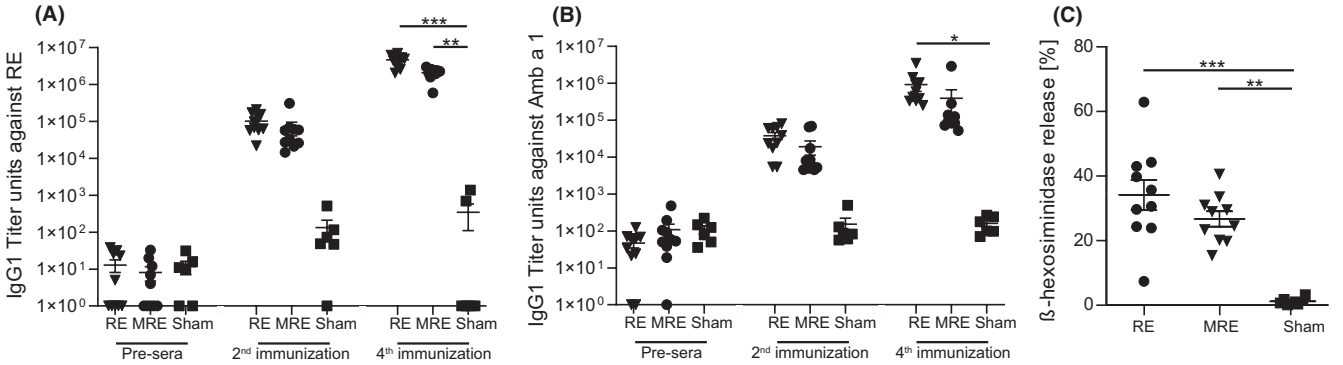

FIGURE 2 Mouse IgG ELISA and mediator release assay (muRBL). IgG against coated RE (A) and coated natural purified Amb a 1 (B) were determined by ELISA. Female BALB/c mice (n = 10 per group) were s.c. injected with 10 µg MRE or with its equivalent RE both adsorbed to $Al(OH)_3$ per mouse on days 0, 7, 14, and 21. Control mice (n = 6) were injected with PBS (with alum) only. Specific IgG titers were determined in single serum samples obtained at days −1 (pre-sera), 13 (2nd immunization), and 28 (4th immunization). C, Mediator release of RBL cells passively sensitized with murine sera obtained at day 28 and stimulated with 100 ng/ml RE. Data represent ß-hexosaminidase release of cells after subtraction of background (0% release) relative to 100% release values observed with 10% Triton X-100. Statistics were calculated by the Kruskal-Wallis followed by Dunn's post-test, respectively. *p < 0.05, **p < 0.01, and ***p < 0.001

with MRE-immunized mice at the representative RE concentration of 100 ng/ml (Figure 2F), indicating similar allergenic potency.

IgE-facilitated allergen binding (FAB) assays were used to determine the capacity of IgG induced upon immunization of mice to prevent IgE-RE complex formation and revealed that IgG induced upon MRE immunization possesses a higher inhibition capacity than IgG induced by RE immunization (Figure S1B).

ELISpot experiments were performed using splenocytes of RE- or MRE-immunized mice collected 1 week after final immunization and restimulated with either RE, MRE, or tissue culture medium to identify induction of inflammatory cytokines. RE and MRE restimulation of splenocytes from mice immunized with either RE or MRE did not induce IL-10–producing cells but significantly increased IFN-γ–secreting cells (in case of RE) (Figure S4A); additionally, IL-4 (Figure S4B)- and IL-5 (Figure S4C)–secreting cells were elevated compared to restimulation with medium. Thereby, RE was a significantly stronger stimulus for the induction of all three cytokines, compared to stimulation with MRE, independent of the immunization regime. Stimulation of splenocytes from mice immunized with sham did not lead to any induction of cytokine-producing cells.

In conclusion, it was demonstrated that chemical modification of ragweed significantly reduced IgE binding and mediator release from human sera about 500-fold, suggesting a significantly decreased allergenicity. Furthermore in mouse models, MRE has proved to be a strong inducer for antibodies, which are cross-reactive with unmodified ragweed pollen proteins. Immunization with MRE induced slightly less total and allergen-specific IgE but at the same time induced higher IgG1 levels. Further, in ELISpot experiments MRE induced significantly less IFN-γ and IL-4 and IL-5, in comparison with RE. Taken together, these results demonstrate that MRE is a highly valuable candidate to be further tested as vaccine for the treatment of ragweed pollen allergies. In future studies using an immunotherapy model, the therapeutic efficacy of MRE will be addressed in detail.

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KEYWORDS

allergoid, immunogenicity, pollen extract, ragweed immunotherapy

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CONFLICT OF INTEREST

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SUPPORTING INFORMATION

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

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Cost-effectiveness of the subcutaneous house dust mite allergen immunotherapy plus pharmacotherapy for allergic asthma: A mathematical model

To the Editor,

Previous research suggests that allergen immunotherapy (AIT) may be cost-effective for the treatment of patients with allergic asthma (AA).[1,2] However, most evidence is based on randomized controlled trials, and the use of policy-relevant outcomes like exacerbations and medication step down is lacking. Potential differences in the cost-effectiveness across populations have also been underexplored.[1,3] Recently, in Allergy Journal Schmitt *et al* reported for the first time from German real-world data that AIT reduced the progression of asthma using GINA steps as severity categories.[4] Also, Jutel et al. reported a 10.8% reduction in the prescription of AA medications and a 59.7% reduction of allergic rhinitis (AR) medications among pediatric patients who received subcutaneous immunotherapy (SCIT).[5] In Colombia, Sánchez et al. reported in a real-world study that 40% of patients with moderate-persistent allergic asthma treated with SCIT and inhaled corticosteroids (ICS) achieved a complete withdrawal of asthma medications after monthly administration of the SCIT during three years. However, no evidence regarding the potential economic implications of the SCIT exists in Colombia and Latin America. Under this same perspective, we evaluated the cost-effectiveness of SCIT plus ICS vs ICS for AA, in pediatric and adult patients with or without AR, from the perspective of the Colombian healthcare system using a mathematical modeling approach and parameters from multiple sources including real-world studies. Model-based cost-effectiveness evaluations are advantageous because they allow the combination of multiple sources of evidence, extrapolation of results beyond the study length of clinical trials and converting treatment effects into policy-relevant outcomes [1,3]

We developed a cohort state-transition model (Markov model) to simulate potential consequences in costs and health-related quality-of-life of the evaluated strategies in the clinical pathway of asthmatic patients over a 10-year time horizon, divided into incremental 3-month cycles (time where changes in health status are expected). Within the model, a hypothetical cohort (ie, a group of individuals that could theoretically receive the evaluated strategies) of 1,000 8-year-old patients (at baseline) per strategy with moderate-persistent AA (sensitized to house dust mites [HDM] with clinically relevant symptoms, as these are the main source of sensitization among