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Russian thistle pollinosis: form allergen characterization to specific immunotherapy treatment

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1. ABSTRACT

Chenopodiacea/Amaranthacea pollen is an important cause of respiratory allergy around the world. The problem could be aggravated because in desert or desert-like countries, these weeds are used in greening programmes or as ornamental plants. As climate tendencies should maintain in future decades, relevance of *Chenopodiacea/Amaranthacea* pollen allergy will go up. Relationship between airborne pollen grains and symptoms may not follow conventional patterns, and a wide lag between pollen counts and symptoms has been observed. Allergens from *Chenopodium album* y *Salsola kali* are probably the best known. A mayor band protein of 40-43 kDa has been associated with the main *Salsola kali* allergen, named sal k 1 and now recognised as pectin methyltranspherase or glucose 3 phosphate dehidrogenase. Systemic immunotherapy is effective and safe, improving symptoms, medication intake and Quality of Life and also reduces cutaneous allergen response.

2. CHENOPODIACEA AROUND THE WORLD

Chenopodiaceae-Amaranthaceae families are ubiquitous plants around the world. They are adapted to many ecological conditions but grow most vigorously in soils rich in nitrogen. It grows from sea level to over 1000 m and in open and shaded sites. Table 1 shows relevant allergenic components of these families.

Although not similar in appearance they are closely related. The inhalation of *Chenopodiaceae* pollen is an important cause of allergic respiratory symptoms in the West and Centre states of the USA, in North Africa, in Mediterranean and some Arabic countries (1-5) . *Amaranthaceae* are very ubiquitous, specially*Amaranthus retroflexus*, and can be found in cultivated or waste soil in America and Europe. These plants, specially tumbleweeds, are also abundant in several semi-desert regions of other countries around the world. The problem could be aggravated because in desert

Table 1. Common potentially allergenic plants of *Chenopodiaceae-Amaranthaceae* families

Genus/Species	Common name
Atriplex halimus	Lenscale
Bassia (Kochia) scoparia	Burning bush, frebush
Chenopodium album	Lamb's quarter
Chenopodium murale	Salt-green
Salsola kali	Russian thistle
Salsola vermiculata	Mediterranean saltwort
Amaranthus deflexus	Low amaranth
Amaranthus muricatus	African amaranth
Amaranthus retroflexus	Rough pigweed

Figure 1. Salsola Kali.

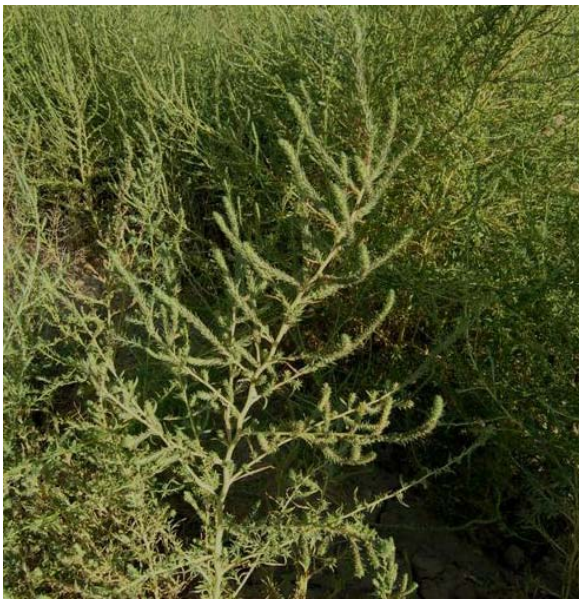

Figure 2. Bassia scoparia.

or desert-like countries, these weeds are used in greening programmes or as ornamental plants (6).

Salsola kali (Figure 1) is a typical plant of salty soils (7), where rainfall is not abundant. The genus *Salsola* is one of the better known genera of the *Chaenopodiacea* family and several species have been described as causes of allergic sensitization, including *S. pestifer*, *S. vermiculata* and *S. soda*. One of the better known species is *S. kali* (Russian thistle). It is a summer flowering, annual weed, which as mature plants forms spherical bushes. Once they start aging, the plant breaks at the soil line and becomes a tumbleweed (8). In this shape, the plant is blown by the wind, spreading between 20 000 and 50 000 seeds. Other important members of these plant families, very frequent in our living area, are *Bassia scoparia* (Figure 2) and *Atriplex halimus* (Figure 3).

In Spain (9), *S. kali* is very common in Toledo, Aragon, Andalucia, Murcia and Levante (10,11,12). In these areas, the pollen load may represent up to 5% of the total pollen, being responsible for many allergic sensitizations (13). In our region (Zaragoza, Spain), sensitisation to *Chenopodiaceae* occupies the second place in the prevalence of sensitisations observed in our outpatient clinic. It affects 42% of the patients with clinical sensitivity to pollens (14). Furthermore, the allergenic incidence of these weeds has been lately going up due to the desertization of extensive zones in Europe.

3. CHENOPODIACEAE POLLEN

Pollen grains of *Chenopodiaceae-Amaranthaceae* families are very similar for all them; however, the plants are very different in appearance. Usually, the pollen of all these plants can't be distinguished by optical microscope. The typical periporate, 20-35 μm diameter with a thin granular exine, is a common image of *Chenopodiaceae-Amaranthaceae* grain pollen (15). The highest pollen counts are detected at the end of August and beginning of September. However, significant pollen counts can be obtained from May to October and patients sensitive to *Chenopodiaceae* may be symptomatic along all this period.

Correlation between natural pollen exposure and allergy respiratory symptoms has been demonstrated in a study conducted by our group (16). We performed a prospective observational study to establish a relationship between pollen counts of *Chenopodiacea/Amaranthacea* and clinical symptoms of rhinoconjuctivitis and asthma in 60 monosensitised patients. All patients collected daily symptom scores during the summer months of 1999, 2000 and 2001. Although many patients experienced symptoms from May to October, we identified a peak of pollen and clinical symptoms in the second half of August and first half of September in the 3 seasons studied. However, correlation between total symptoms and daily pollen grains/m^3 was poor or absent (16). Symptoms score curve was lagged from pollen counts curve almost two weeks. After further analysis (trough time-series tests), and by displacing one of both curves between 11 to 17 days, the correlation coefficients for total symptoms, improved for 1999 ($r = 0.744$; $p < 0.0001$) and became significant for 2000 ($r = 0.521$; $p < 0.0001$) and 2001 ($r = 0.635$; $p < 0.0001$) (16). Figure 4 shows a simplified representation of this relationship: pollen curve and total symptoms score curve fits very well if displacing 15 days one to another. So, we identified a significant time lag between pollen counts and symptom scores in *Chenopodiacea/Amaranthacea* monosensitized patients.

There is no simple explanation for these findings. The correlation between symptom scores and pollen counts

Figure 3. Atriplex halimus.

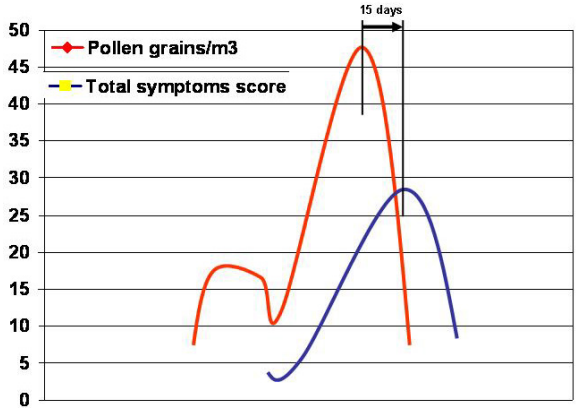

Figure 4. Schematic representation of relationship between chenopodiacea pollen counts and symptoms. The total symptoms score curve lags 15 days the pollen counts curve. More details in reference 16.

could be hampered by the fact that in all aerobiological surveys, the pollen of several *Chenopodiacea* and *Amaranthacea* species are grouped together, since they cannot be recognized individually. For example in Zaragoza, pollen grains of *Amaranthus albus*, *A. retroflexus*, *Atriplex halimus*, *A. portulacoides*, *Bassia scoparia*, *Chenopodium murale*, *C. album*, *Salicornia europaea*, *Salsola kali* and *S. vermiculata* are grouped together (14). In this area, *B. scoparia* seems to be frequently present in urban areas, whereas *S. kali* and *S. vermiculata* are predominant in the surrounding rural areas (17). Although variable degrees of cross-reactivity have been suggested between *Chenopodiacea* and *Amaranthacea* species (18, 19), the allergenicity of many of these species present in our living area has not been evaluated.

Other possible reason for this lag could be that there is a complex, nonlinear relationships between pollen counts and symptoms, that seem to reflect both the priming effect and late-phase reactions. A significant correlation between air pollution and symptom scores has also been demonstrated (20), and it has been suggested that allergens, carried on pollen grains, or on plant-derived paucimicronic components, may interact with air pollution. Airway mucosal damage and impaired mucociliary clearance induced by air pollution may facilitate the access of inhaled allergens to the cells of the immune system, thus influencing the overall symptom scores.

4. SALSOLA KALI ALLERGENS

As mentioned before, the genus *Salsola* is one of the better known genera of the *Chaenopodiacea* family. Sal k 1, an important allergen in *S. kali* has been recently described. This allergen was characterized using serum samples from Zaragoza's area (21). A 43 kD prominent band protein was identified by SDS-PAGE an then it was purified. Other predominant bands were identified at 15, 17, 18, 29, 58, 69, 74 and 97 kDa. The allergenic profile was studied by immunoblot. Bands that were more frequently recognized had molecular weigh between 36 and 90 kDa, especially those with molecular weigh of 43, 57, 69 and 81 kDa. A total of 20 patients and 10 controls were skin tested with the 43kDa purified protein that elicited a higher cutaneous response than native extract. However, the N-terminal of the purified protein was blocked and then, the protein was fragmented with trypsin to obtain internal peptide sequences. The sequence of the four fragments did not show any homology with other known proteins and the allergen was registered at the SWISS-PROT data bank, in the allergen data bank, as Sal k 1 (21).

Recently, a 40 kDa band protein has been investigated again and a sequence has been more accurately established (22). The comparison of the sequencing data from the peptides obtained after trypsin digestion with the protein sequences of the data banks, allowed the authors to detect homology with plant pectin methylesterase (PME) family. The isolated protein was recognized by the pool of sera in immunoblotting, and it is able to almost completely inhibit the IgE binding to the 40 kDa band. Further analysis of the same protein band showed up to eleven different proteins identified trough mass spectrometry. However, most of them are isoforms of pectin methyltranspherase and glucose 3 phosphate dehidrogenase (23).

Cross-reactivity within the *Chenopodiaceae* family is a common feature of patients suffering from respiratory allergy to these pollens. In fact, high cross reactivity between *Chenopodium album* and *S. kali* is well known (18). Furthermore, in other species taxonomically less related as members of the *Amaranthaceae* family (i.e. *Amaranthus retroflexus*), high cross reactivity has been reported for patients suffering sensitization to *Chenopodiaceae* pollens (19). Correlations of the sensitivity against *C. album* in patients sensitized to *S. kali* have been reported to vary between 70–96% and 70–75% by skin prick test (SPT) or RAST, respectively (10,24). Furthermore, *Chenopodium album* allergens have been recently characterized and three major allergens have been recognized (6). Che a 1, is a glycoprotein whose sequence exhibits 27-45% identity with known members of the Ole e 1-like protein family and a recombinant form has been expressed in yeast (25). More than 75% of sera from patients allergic to chenopod pollen were reactive to Che a 1 (26). Che a 2 (a profilin) and Che a 3 (a polcancin) has

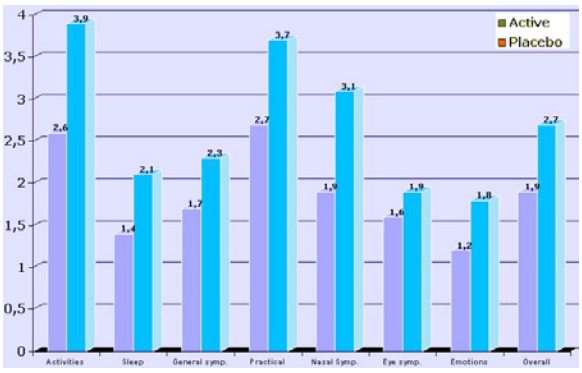

Figure 5. RQLQ in the season after 1 year treatment with a modified therapeutic vaccine of Salsola kali, at the end of the study in active and placebo groups. Overall and all domains score, but eye symptoms, had a difference higher than 0,5 points favoring active treatment. *p<0,05. More details in reference 29.

also been characterized and may play a major role in cross-reactivity between *Chenopodiaceae* (27). Recombinant Che a 2 (rChe a 2) has been produced in Escherichia coli cells by the same authors (28). However, Sal k 1 is considered an allergen responsible for the different sensitization between *S. kali* and *C. album* pollen, so it may be a useful marker to classify patients allergic to *Chenopodiaceae* (22).

5. IMMUNOTHERAPY WITH A SALSOLA POLLEN EXTRACT

Although the inhalation of *Chenopodiaceae* pollen is a well known common cause of respiratory diseases in Europe and North America from many decades ago, well documented studies regarding specific immunotherapy efficacy has only recently appeared (29). A previous approach in PhD thesis by de la Hoz (30), supports the clinical benefit of this treatment. More recently, an article was published on the safety of a physically modified extract (adsorbed to alum) (31). Taking this background into account, our group conducted a new study to evaluate the clinical efficacy and safety of a vaccine containing a chemically modified (depigmented and glutaraldehyde polymerized) extract of *Salsola kali* in a group of patients sensitized to this pollen (29).

Sixty patients selected in our clinic completed the study. All they were sesitizated to *Salsola kali* and no other allergen besides *Chenopodiaceae* pollen. They were randomly distributed to receive the extract of *Salsola kali* or placebo during one year. All patients recorded daily symptom scores and medication use on diary cards during the pollen season. Dose-response skin tests were performed at baseline and at the end of the trial. Nasal challenge test was also performed before treatment start and at the end of the study. A Rhinoconjunctivitis Quality of Life Questionnaire was completed in the previous pollen season (before treatment) and during the pollen season 1 year later (in the trial). Dose-response skin tests were performed at baseline and at the end of the trial (29).

The active treated group had fewer symptoms and lower intake of medication during the pollen season with a significant difference ($P < 0.05$) compared with placebo receiving group. There was a fifty percent more reduction of symptoms (thirty percent in medication intake) in active treated group than in placebo one (29). Both measures exceed the values for clinical relevance recently recommended (32). However, when symptoms were individually analyzed, this difference was significant for nasal and bronchial symptoms and not significant for ocular symptoms. Although this study was not powered to assess efficacy on asthma, there were 19 patients with asthma in the sample: 6 in the placebo and 13 in the active group. With the limitations mentioned, we detected significant differences in asthma symptoms between groups, favouring active treatment (29). The number of days without symptoms was higher in the active group too ($P < 0.05$). These actively treated patients also had a significant improvement in the Rhinoconjunctivitis Quality of Life Questionnaire ($P=0.017$) and a reduction in skin sensitivity ($P=0.03$). The lack of significant improvement in ocular . Nasal challenge response was also significant reduced in the immunotherapy treated patients but not in those received placebo (unpublished data). symptoms was also corroborated in the quality of life questionnaire, which suggests a good correlation between symptom scores and quality of life issues. Consistent with previous analyses, a change in score greater than 0.5 on the RQLQ domain and overall scores was the clinically meaningful minimal important difference (33). In our study (29), this change was obtained for all domains of the RQLQ in the active group, when comparing the season before with de season after the treatment, and almost all domains at the end of study when comparing active with placebo group (Figure 5). A visual scale of health situation related to symptomatology (34) also supported these results and showed a significant difference ($P < 0.001$) with a forty percent more reduction in active group vs. placebo group. A significant reduction in skin test was also observed only in the active treatment group (29).

Regarding safety, the treatment was well tolerated, and no severe systemic reactions or serious adverse effects were recorded. The local reactions were all delayed, small, and clinically not relevant (29). So, we concluded that Immunotherapy with this modified vaccine of *S. kali* pollen is safe and efficacious to treat patients clinically sensitive to this pollen.

At the end of this study's phase patients follow up continued in an open mode. Patients on active treatment kept up their treatment with extract (chemically modified, depigmented and glutaraldehyde polymerized) of *Salsola kali* for two more years. In the other hand, patients on placebo started then immunotherapy with a conventional, biologically standardized and alum adsorbed *Salsola kali* extract, through a cluster build up and then continued treatment for three years. Patients follow up was conducted like the first study phase. Data analyses are still ongoing, but preliminary results suggest that patients incorporated on alum adsorbed *Salsola kali* extract, improve clinical parameters almost like patients on chemically modified

(depigmented and glutaraldehyde polymerized) extract did (35).

6. FUTURE RESEARCH

As climate tendencies should maintain in future decades, relevance of *Chenopodiacea/Amaranthacea* pollen allergy will go up. Notable advances in knowledge of these plants allergens have been recently published and other proteins with different molecular weigh should be investigated. Furthermore, the relevance of different species of *Chenopodiacea/Amaranthacea* will be explored. Optical aspect of their pollen is very similar, but allergenic content of it should be studied for many clinically important species of each area. Other forms of immunotherapy (i.e. sublingual) should also be investigated.

7. CONCLUSIONS

Chenopodiacea/Amaranthacea pollen is an important cause of respiratory allergy around the world. Relationship between airborne pollen grains and symptoms should be investigated in depth. Systemic immunotherapy is effective and safe, improving symptoms, medication intake and Quality of Life.

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Key words: Chenopodiacea, *Salsola kali,* Russian thistle, Allergic rhinitis, Rhinoconjunctivitis, Quality of Life, Visual analog scale, Immunotherapy, Review

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