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The molecular allergology of subtropical grass pollen

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ABSTRACT

Grass pollens are amongst the most important aeroallergen sources world-wide triggering allergic rhinoconjunctivitis and asthma in sensitised patients. Much of what we know about the allergen components of grasses is informed by research on pollen of temperate (Pooideae) species that are abundant in the temperate climate zones. However, climate changes are altering the biogeographical distribution as well as timing and allergenicity of grass pollens. This provides an impetus for better understanding of the contribution of subtropical subfamilies of grasses to pollen allergy globally. Pollen of Chloridoideae (e.g. *Cynodon dactylon*; Bermuda grass) and Panicoideae (e.g. *Paspalum notatum*; Bahia grass or *Sorghum halepense*; Johnson grass) subfamilies are clinically important in subtropical zones of Australia, Asia, India, Africa, and America. These grasses differ ecologically and phylogenetically from temperate grasses and, importantly their allergen composition is qualitatively different. For example, subtropical grass pollens appear to lack the major group 5 grass pollen allergen family. In this review we summarize current knowledge of the epidemiology and immunology of subtropical Chloridoideae and Pancoideae pollen allergens, describe the biochemical characteristics of known isoforms and variants as well as properties and structures of subtropical pollen allergen components. Whilst only one subtropical allergen component; Cyn d 1 of Bermuda grass pollen, is available commercially for diagnostic use, in a natural purified form, a number of allergens of Panicoideae grass pollen; Zea m 1, Zea m 3 and Zea m 13 of maize, Pas n 1 and Pas n 13 of Bahia, as well as Sor h 1, Sor h 2, Sor h 13 and Sor h 23 of Johnson grass, have been discovered. Research effort is directed towards making available subtropical grass pollen allergen components as innovative treatment and diagnostic options that more specifically address the needs of patients from warmer regions of the globe.

1. Biogeography, epidemiology and immunology of subtropical grass pollen

There is an inverse biogeographical distribution of temperate and subtropical grasses with the subtropical species being more abundant closer to the equator (Esch, 2004). The size of the world's population living in subtropical climates is increasing globally and the subtropical climate zones are widening (Gupta, 2002; Seidel et al., 2008). In southern United States of America (USA), such as Florida, Texas, Louisiana and Mississippi, the population increased by 18.3%–52.3 million between 2000 and 2010 (US Census Bureau). The biomass of subtropical grasses (Morgan et al., 2011) and their range is predicted to expand with climate change (Gupta, 2002), increasing the exposure to subtropical GP allergens and intensifying the burden of allergic respiratory diseases (Beggs, 2009; Ziska and Beggs, 2011). The epidemiology of subtropical grass pollens and their contribution to allergic rhinoconjunctivitis and asthma in subtropical regions has previously been reviewed (Davies, 2014). Here a comparison between temperate and subtropical grass pollen biogeography, epidemiology and immunology is summarized.

In 1972, Hensel and Griffith (1972) examined sensitisation frequencies in the 429 patients from Louisiana, a subtropical region in USA; Bahia (*Paspalum notatum)* GP was the most frequently recognized GP but the patterns of sensitization included SPT positivity to Bahia, Dallis (*Paspalum dilatatum*), Johnson (*Sorghum halepense*), Bermuda (*Cynodon dactylon*) and Timothy (*Phleum pratense)* GP. Application of the Prauss Kausner test (Cohen and Zelaya-Quesada, 2002) with serum of five Bahia GP-allergic patients to non-allergic recipients, showed higher SPT reactivity to Bahia than other species of GP (Hensel and Griffith, 1972).

A survey of sensitisation of 345 children of military personnel with allergic diseases in Lackland, Texas, Bahia GP showed the highest frequency (38%) of positive SPT of a panel of 51 common aeroallergens (Calabria and Dice, 2007). Interestingly, the frequency of sensitivity to

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pollens of other grasses; Bermuda (35.9%), Ryegrass (*Lolium perenne*) (34.8%) and Timothy (34.2%), were only slightly lower. The authors considered the study cohort to be from a "mobile" population and it included children up to 18 years of age. There was an increase in prevalence of allergic sensitivity with age, therefore it was unclear whether sensitisation to GP occurred whilst the children lived in Texas and were exposed to grasses of Texas.

Examination of serum IgE reactivity to GP in allergy patients from Europe and North America revealed high correlations between specific IgE to various GP in single point tests (Andersson and Lidholm, 2003; Johansen et al., 2009). However, the degree of correlation between specific IgE concentrations to subtropical GP and temperate GP was not as high as amongst temperate GP. Most of the patient sera examined in those studies were sourced from patients in regions primarily exposed to temperate GP.

IgE cross-inhibition assays can be used to indicate the avidity of interaction based on the inhibitor concentration at which 50% (IC50) of IgE reactivity with the target allergen is blocked, and the degree of specificity of response (maximum IgE cross inhibition) (Aalberse, 2007). IgE cross reactivity between subtropical and temperate GP is incomplete and mostly non-reciprocal, depending on the origin of the patient and their exposure to subtropical and/or temperate GP allergens (White and Bernstein, 2003; Weber, 2003; Davies et al., 2011a; Davies et al., 2012). The capacity for the immune system to differentiate between allergens of temperate and subtropical GP at the T and B cell level has important implications for the specificity of GP allergy diagnosis and the likely efficacy of GP allergen specific immunotherapy (Nony et al., 2015; Burton et al., 2002; Etto et al., 2012; Eusebius et al., 2002).

In a cross-inhibition study from Minnesota, USA, with pooled sera of five donors highly allergic to northern GP, Bermuda GP was unable to inhibit 50% of IgE with June (*Koeleria macrantha*), orchard (*Dactylis glomerata*), meadow fescue (*Festuca pratensis*) or Ryegrass GP. In the converse experiments, four orders of magnitude more Timothy GP extract was required to achieve 50% inhibition of IgE with Bermuda GP (Leiferman and Gleich, 1976).

Similarly, northern GP showed reciprocal cross-inhibition of IgE reactivity with an array of temperate GP in studies with pooled sera from US army volunteers (Martin et al., 1985). However, Bermuda and Bahia GP extracts showed limited capacity to inhibit pooled serum IgE reactivity with pollens of Ryegrass and Timothy GP extracts in radio allergosorbant assays. Pollen of "northern" grasses inhibited most but not all IgE reactivity with Bahia grass, suggesting "unique allergenicity" for Bahia compared with a temperate GP. Although the origin of the subjects and their primary exposure to temperate or subtropical grasses was not described, distinct IgE reactivity between subtropical and temperate GP were evident.

Small numbers of patients from Florida showed no *in vivo* cross-reactivity between pollen of Timothy and Bahia GP in nasal provocation tests in patients with allergic sensitivity to either Timothy and Bahia GP (Phillips et al., 1989).

Recently, Ramirez et al. (2015) used an allergen challenge chamber in Texas to investigate sensitivity to Timothy GP where subtropical grasses predominate without natural Timothy GP exposure. Of the 22 participants, SPT and specific IgE to Timothy and subtropical Bahia, Johnson and Bermuda GP correlated with symptom scores following exposure to Timothy GP in the allergen challenge chamber. The authors did not observe differences between symptom scores of participants who were locals of Texas and those who had lived outside of Texas for over 5 years. However, at excessively high allergen exposure in the chamber (over 3000 grains per meter cubed over three hours) those participants who were local Texans showed slower kinetics of symptom escalation than those who had been exposed to Timothy GP outside Texas suggesting differences in allergic sensitivity between the two subgroups of participants (Ramirez et al., 2015).

In different states of Australia, Davies et al. (Davies et al., 2011a; Davies et al., 2012) showed that levels of specific IgE reactivity with subtropical and temperate GP differ depending on the biogeographical origin and grass exposure patterns of the patient. Moreover, the finding of specific IgE recognition of subtropical species of GP in patients from subtropical Queensland was subsequently confirmed in separate studies (Nony et al., 2015).

2. Biology and biochemistry of subtropical GP allergens

GP allergen families include proteins with particular biochemical structures and functions within the pollen from which they are derived. The allergens discovered within subtropical GP include the group 1; β-expansin, group 13; polygalacturonase, and others described below. Notably, the highly allergenic group 5 allergen of temperate GP does not occur in subtropical grass pollens. Table A1 summarizes the subtropical GP allergens identified to date for which there is evidence of patient IgE reactivity or allergenicity. Proteomic analysis of Bermuda GP revealed eight allergens that shared similarities to known pollen allergen families based on mass spectrometry and databases comparisons (Kao et al., 2005). Analysis of the complete proteome, transcriptome and allergome of Johnson GP, demonstrated that there are more gene transcripts present within the pollen that encode for allergen-like proteins, and more detectible isoforms translated into proteins and packaged within pollen, than there are IgE-binding proteins detected with serum of relevant, clinically-affected allergy patients. Thus the allergen status of putative allergens identified by molecular biological techniques must be verified (Pomes et al this issue).

2.1. Major allergens: group 1

The major Group 1 GP allergens, β-expansins constitute up to 10% of total pollen (Drew et al., 2011). Functionally, these non-proteolytic glycoproteins are involved in the loosening of the cell walls to facilitate invasion of the pollen tube (Cosgrove et al., 1997). In molecular and biochemical studies of the maize (*Zea mays*) allergen Zea m 1, it was proposed that β-expansins loosens plant cell walls by disrupting noncovalent junctions between the matrix polysaccharide glucuronoarabinoxylan, that binds cellulose (Wang et al., 2016). Cyn d 1, from Bermuda GP, was the first group 1 allergen to be characterized as a 32 kDa protein with high N-terminal sequence homology to the well characterized group 1 allergen of Ryegrass, Lol p 1 (Shen et al., 1988; Matthiesen et al., 1991). Group 1 allergens of subtropical GP show the highest frequency of IgE reactivity by immunoblotting in GP allergic patients (Davies, 2014), ranging from IgE reactivity to Cyn d 1 in 76% of 21 patients from Taiwanese and 100% of 44 patients from New South Wales, Australia (Shen et al., 1988; Ford and Baldo, 1987). A 33 kDa acidic (pI 6.59) allergen purified biochemically from Bahia GP showed IgE reactivity with sera from patients in Florida USA (Ghobrial et al., 2002). The Bahia GP Group 1 allergen of 29 kDa was subsequently cloned, Pas n 1 (Davies et al., 2008; White et al., 2009). By ELISA, recombinant Pas n 1 showed IgE with 47 of 55 (85%) patients from the temperate climate city of Melbourne Australia (Davies et al., 2008) and by ImmunoCAP purified Pas n 1 showed IgE reactivity with 91.2% of 182 GP-allergic (Timbrell et al., 2014). Sor h 1 was identified (Avjioglu et al., 1993) and latter characterized as 30 kDa protein that showed by ELISA IgE reactivity with 76% in sera of 64 patients from Queensland, Australia (Campbell et al., 2015).