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Antigenic cross-reactivity between *Schistosoma mansoni* and pollen allergens from the birch tree (*Betula verrucosa*) and Timothy grass (*Phleum pratense*): involvement of shared glycan epitopes and implications for the hygiene hypothesis

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ABSTRACT

Previous studies have shown that schistosome infection can protect against allergic symptoms, but the underlying mechanisms are still not fully understood. Here we have shown that rabbit IgG antibodies raised against *Schistosoma mansoni* soluble egg antigens (SmSEA) are cross-reactive with a wide array of molecules in Timothy grass pollen (TGP) and birch tree pollen (BTP). Five of the cross-reactive pollen molecules (two from TGP and three from BTP) were selected randomly and identified by tandem mass spectrometric (TMS) analysis to be, respectively, the TGP allergens Phl p 1 and Phl p 5b, and BTP glutathione S-transferase (GST), and the BTP allergens Bet v 1 and Bet v 6.0102. Rabbit anti-SmSEA IgG antibodies that cross-reacted with each of the five allergens were found to be reactive with three major *S. mansoni* egg antigens, IPSE/alpha-1, omega-1 and kappa-5. Pairwise alignment of the amino acid sequences of each of the five TMS-identified pollen allergens with each of the three egg antigens revealed a low level of amino acid sequence identity. Further experiments indicated that the schistosome antigen/allergen cross-reactivity was mostly due to similar glycans present in helminths and plants, but not in mammals: so called cross-reactive carbohydrate determinants (CCDs). Previously, CCDs have been implicated in the cross-reactivity between many plants and invertebrates. Furthermore, pollen-induced anti-CCD IgGs have been found in sera of patients undergoing allergen-specific immunotherapy (SIT) and implicated in the treatment of the allergy. Thus, our finding provides not only possible explanations for the allergy-protective effect of helminth/schistosome infections as explained by the hygiene hypothesis, but also a potential starting point for improved SIT.

1. Introduction

In recent decades populations benefitting from well-developed health systems have suffered from a steep rise in the occurrence of allergies, asthma and other disorders of the immune system of humans (Bach, 2002). The 'hygiene hypothesis' is often offered as an explanation, i.e., due to an increase in healthy living conditions and/or prevention of infection by vaccinations and drugs, populations have suffered less from parasitic and microbial infections and the patterns of maturation of human immune systems are therefore now different from those that pertained to pre-hygienic, infection-rife eras (Rook and Stanford, 1998). A consequence of this difference is an untimely and pathological immune response to environmental and air-borne antigens that are responsible for asthma and allergies, and which 'normal' human immune responses would have perceived as innocuous. The hygiene hypothesis may also have relevance to autoimmune and inflammatory diseases such as inflammatory bowel disease and Type 1 diabetes (Zaccone et al., 2006; Koloski et al., 2008). In helminth-endemic countries in particular, an inverse correlation has been observed between infection with helminths including schistosomes, and sensitisation to allergies (Yazdanbakhsh and

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Wahyuni, 2005; Rujeni et al., 2012), for which situation the hygiene hypothesis has been evoked as an explanation (Rook, 2007).

Immune responses that cause allergic reactions and that are also induced by helminth infections are so-called Th2-type responses (Steinke et al., 2008; Allen and Maizels, 2011). These are mostly characterised by involvement of Th2 cells, the production of IgE antibodies, the activation of granulocytes such as mast cells, basophils and eosinophils, and the release of inflammatory factors such as histamine and cytokines such as IL-4, IL-5, and IL-13 (Steinke et al., 2008; Allen and Maizels, 2011). When helminth infections advance to chronicity, Th2-type responsiveness may become modulated or modified and characterised by the activities of regulatory T- (Treg) and B-cells, and the production of cytokines such as IL-10 and TGF-β (Caldas et al., 2008). The immunomodulated state may facilitate parasite survival and possibly also that of the host (van Die and Cummings, 2006).

The above-mentioned immunomodulatory mechanisms by which parasites down-regulate excessive helminth-induced Th2 responsiveness have been reported to have a suppressive effect on allergic sensitisation (Schnoeller et al., 2008). The processes by which this occurs have not been fully elucidated, although one widely accepted explanation pertinent to the hygiene hypothesis is that chronic helminth infection, for example with *Schistosoma mansoni*, results in the activation of Treg cells and production of cytokines such as IL-10 and TGFβ (Araujo et al., 2004). IL-10 and TGF-β are anti-inflammatory cytokines which may have a dampening effect on atopic diseases such as allergy and asthma (Araujo et al., 2004).

So-called 'blocking antibodies' may provide an alternative explanation to account for the negative correlation between helminth infection and allergy. Helminth infections induce the production of copious amounts of IgE, much of which probably lacks the function of specific antibodies, but it could saturate Fcε receptors on mast cells and basophils, and thus 'block' the cross-linking of such receptors by allergen-induced specific IgE antibody (Araujo and de Carvalho, 2006). There is, however, evidence against this possibility (Mitre et al., 2005).

On the other hand, many proteins in plants and invertebrates including helminths are glycoproteins and the glycanic residues have been reported to be major targets of IgE antibodies that are induced after an insect sting or exposure to plant pollens or food (Tretter et al., 1993; Aalberse and van Ree, 1997; van Ree et al., 2000; Kwaasi et al., 2002; Bencurova et al., 2004; Wicklein et al., 2004, Paschinger et al., 2009). These glycan epitopes are present in identical form in many different plants and invertebrates and have been termed cross-reactive carbohydrate determinants (CCDs) (van Ree, 2002, Wicklein et al., 2004). IgE anti-CCD antibodies are, however, generally considered to be of little clinical relevance (Altmann, 2016).

We have recently begun to investigate the antigenic cross-reactivity between *S. mansoni* and allergens from different organisms, and have to date demonstrated cross-reactivity between the helminth's molecules and the allergens Hev b 7 from latex (Doenhoff et al., 2016), and Ara h 1 from peanut (Igetei et al., 2017). Because pollen allergens are of considerable public health importance as agents of allergic sensitisation in humans and reported to trigger IgE sensitisation in ~40% of all allergic patients (Freidhoff et al., 1986; Stumvoll et al., 2002), we have extended the aforementioned studies to an exploration of the antigenic relationships between *S. mansoni* soluble egg antigens (SmSEA) and allergens from birch tree pollen (BTP, *Betula verrucosa*) and Timothy grass pollen (TGP, *Phleum pratense*).

To identify cross-reactive antigens/allergens we used rabbit IgG antibodies reactive against *S. mansoni* egg antigens (anti-SmSEA) to probe western immunoblots carrying aqueous-soluble constituents of BTP and TGP. Five cross-reactive antigens from the pollen extracts, three from BTP and two from TGP, were purified separately by means of SDS–PAGE and analysed in tandem mass spectrometry (TMS). The rabbit anti-SmSEA IgG antibodies that were cross-reactive with each of the five purified pollen allergens were purified separately by acid elution from immunoblots carrying each of the antigenically cross-reactive allergen molecules. The acid-eluted antibodies were used to probe immunoblots of SmSEA to identify the *S. mansoni* egg antigens that may have induced production of the allergen cross-reactive antibodies in the rabbits.

Some patients suffering from allergic rhinitis towards pollen are unable to eat fruits or vegetables implicated in the so-called 'oral allergen syndrome' (OAS) (Kwaasi et al., 2002; Cadot et al., 2003). Therefore, the reactivity of the eluted anti-SmSEA IgG antibody preparations eluted from the five pollen molecules was tested on extracts of five plants implicated in OAS. The characteristics of epitopes involved in the cross-reactivity between the antigens of *S. mansoni*, the two pollen extracts and constituents of the five plants involved in OAS were investigated for the involvement of homologous peptides and/or CCDs using bioinformatics and the effects of treatment with a mild solution of sodium metaperiodate.

The possibility that anti-*S. mansoni* IgG antibodies that are cross-reactive with allergens could somehow block allergic reactions is discussed as a possible explanation for the hygiene hypothesis.

2. Materials and methods

Except when stated otherwise, all chemicals, reagents and buffers used in all techniques are of analytical grade and were purchased from Sigma–Aldrich, Poole, Dorset, England.

2.1. Preparation of SmSEA extracts

The life cycle of a Puerto-Rican isolate of *S. mansoni* was maintained by continuous passages through the aquatic snail *Biomphalaria glabrata* and CD1 strain random-bred mice. Experiments that used mice for production of *S. mansoni* antigens and rabbits for production of antisera were approved by the Ethical Review Committee of the universities in which these materials were produced and the work was carried out in strict accordance with the regulations set out in the UK Animals (Scientific Procedures) Act, 1986, (Project licence numbers PPL 40/3024 and 40/3595). Animals were euthanised using a lethal dose of pentobarbitone anaesthetic.

SmSEA were extracted from eggs from the livers and intestines of infected mice harbouring adult worms as previously described by Doenhoff et al. (1988). The soluble extract was split into 1 mg aliquots in 0.5 ml Eppendorf vials prior to lyophilisation, and stored at −80 °C until use.

2.2. Preparation of pollen and other plant allergen extracts

Soluble extracts of BTP and TGP were prepared by incorporating 50 mg of each pollen powder in 1 ml of 0.05 M Tris–HCl buffer, pH 7.5, and extracted as described previously (Schenk et al., 2009). The soluble extracts obtained after centrifugation were split into 100 µl aliquots in 0.5 ml tubes and stored at −20 °C until use.

Peanut (*Arachis hypogaea*) extract was prepared from fresh peanut seeds bought from a grocery shop and protein extraction was done as described previously (Doenhoff et al., 2016).

All other fruits used for antigen extraction, namely melon (*Cucumis melo* spp.), banana (*Musa* spp.), tomato (*Lycopersicon esculatum*) and kiwi fruit (*Actinidia deliciosa*) were bought ripe from grocery stores and were prepared in the same way as described for peanut.

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