International Journal for Parasitology xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

International Journal for Parasitology

journal homepage: www.elsevier.com/locate/ijpara

# *Amblyomma sculptum* tick saliva: α-Gal identification, antibody response and possible association with red meat allergy in Brazil

Ricardo Nascimento Araujo [a], Paula Ferreira Franco [a], Henrique Rodrigues [a], Luiza C.B. Santos [a], Craig S. McKay [b], Carlos A. Sanhueza [b], Carlos Ramon Nascimento Brito [a], Maíra Araújo Azevedo [a], Ana Paula Venuto [a], Peter J. Cowan [c,d], Igor C. Almeida [e], M.G. Finn [b], Alexandre F. Marques [a,*]

[a] *Universidade Federal de Minas Gerais, Instituto de Ciencias Biologicas, Departamento de Parasitologia, Pampulha, Belo Horizonte, Minas Gerais, Brazil*
[b] *Georgia Institute of Technology, School of Chemistry and Biochemistry, Atlanta, GA, USA*
[c] *Immunology Research Centre, St. Vincent's Hospital, Fitzroy, Melbourne, VIC 3065, Australia*
[d] *Department of Medicine, University of Melbourne, Parkville, VIC 3052, Australia*
[e] *Border Biomedical Research Center, Department of Biological Sciences, University of Texas at El Paso, El Paso, TX 79912, USA*

ARTICLE INFO

*Article history:*
Received 8 November 2015
Received in revised form 4 December 2015
Accepted 10 December 2015
Available online xxxx

*Keywords:*
*Amblyomma sculptum*
Alpha-Gal
Tick saliva
IgE
Red meat allergy

ABSTRACT

The anaphylaxis response is frequently associated with food allergies, representing a significant public health hazard. Recently, exposure to tick bites and production of specific IgE against α-galactosyl (α-Gal)-containing epitopes has been correlated to red meat allergy. However, this association and the source of terminal, non-reducing α-Gal-containing epitopes have not previously been established in Brazil. Here, we employed the α-1,3-galactosyltransferase knockout mouse (α1,3-GalT-KO) model and bacteriophage Qβ-virus like particles (Qβ-VLPs) displaying Galα1,3Galβ1,4GlcNAc (Galα3LN) epitopes to investigate the presence of α-Gal-containing epitopes in the saliva of *Amblyomma sculptum*, a species of the *Amblyomma cajennense* complex, which represents the main tick that infests humans in Brazil. We confirmed that the α1,3-GalT-KO animals produce significant levels of anti-α-Gal antibodies against the Galα3LN epitopes displayed on Qβ-virus like particles. The injection of *A. sculptum* saliva or exposure to feeding ticks was also found to induce both IgG and IgE anti-α-Gal antibodies in α1,3-GalT-KO mice, thus indicating the presence of α-Gal-containing epitopes in the tick saliva. The presence of α-Gal-containing epitopes was confirmed by ELISA and immunoblotting following removal of terminal α-Gal epitopes by α-galactosidase treatment. These results suggest for the first known time that bites from the *A. sculptum* tick may be associated with the unknown etiology of allergic reactions to red meat in Brazil.


## 1. Introduction

Ticks are efficient ectoparasites that feed on a wide range of hosts such as mammals, reptiles, birds and amphibians. During blood feeding, ticks may introduce many pathogenic microorganisms such as protozoan parasites, viruses or bacteria (Ramamoorthi et al., 2005; Randolph, 2009). In addition, the many salivary proteins introduced by ticks into their host can inhibit hemostasis, decrease inflammation processes and modulate the immune system (Brossard and Wikel, 2004; Valenzuela, 2004; Francischetti et al., 2009; Kotal et al., 2015). The effects of tick bites on the immune response are still poorly understood. Here we focus on the production of IgE antibody in response to the cross-reactive carbohydrate determinant galactose-α-1,3-galactose-β-1,4-*N*-acetylglucosamine (Galα1,3Galβ1,4GlcNAc, or Galα1,3LacNac) as a potential mediator of red meat allergy (Commins et al., 2009). This antigen is found in glycoproteins and glycolipids of many mammalian species as well as other organisms, but it is not present in Old World non-human primates and humans due to the loss of the α1,3-galactosyltransferase (α1,3-GalT) responsible for its synthesis (Galili et al., 1987; Galili and Swanson, 1991). Therefore, the α-galactosyl (α-Gal) epitope constitutes a potent non-self marker in human immunology and is a major cause of xenotransplant rejection (Galili, 2005).

Animal models of food allergy have been explored in attempts to clarify mechanisms of sensitization to food proteins; much attention has been focused on the immune response associated with the production of antigen-specific immunoglobulin IgE and hypersensitivity responses upon allergen challenge (Berin and

* Corresponding author at: ICB, UFMG, Av Antonio Carlos 6627, Pampulha, Belo Horizonte, Minas Gerais, Brazil. Tel.: +55 31 3409 2860; fax: +55 31 3409 2970.
*E-mail addresses:* amarques@icb.ufmg.br, marques.af@outlook.com (A.F. Marques).

http://dx.doi.org/10.1016/j.ijpara.2015.12.005
0020-7519/

Mayer, 2009). An α-1,3-galactosyltransferase knockout (α1,3-GalT-KO) mouse has been developed and used primarily in the xenotransplantation field (Thall et al., 1995; Tearle et al., 1996). These animals are also of interest for studies of carbohydrate immunogenicity, since exceptionally high titers of anti-α-Gal IgG antibodies can be elicited upon immunisation (Abdel-Motal et al., 2009a,b, 2010). They therefore represent a unique model among the non-human primates, being similar to humans in the context of α-Gal epitope expression. Here we describe the use of this animal model to test the generation of anti-α-Gal antibody production by both natural (tick saliva containing α-Gal epitopes) and unnatural (bacteriophage Qβ virus-like particles (Qβ-VLPs) displaying the α-Gal epitope) sources. The latter experiments relate to both the use of carbohydrate-bearing VLPs as analytical reagents and as immunogenic platforms for the display of tumour-associated carbohydrate antigens (Yin et al., 2013).

The Galα1,3LacNac epitope was recently identified in the intestinal tract of the European tick *Ixodes ricinus*, and this finding was correlated with red meat allergy in Sweden (Hamsten et al., 2013a,b). Although allergic reactions to red meat are not common, cases of allergic late phase reaction in patients with IgE to the α-Gal epitope have recently been reported in the U.S. (Commins et al., 2011; Commins and Platts-Mills, 2013), Australia (Van Nunen et al., 2009), Germany (Jappe, 2012), France (Morisset et al., 2012), and Japan (Sekiya et al., 2012). In 2006, patients also were reported with severe anaphylactic reactions induced by high IgE antibody titers against the monoclonal antibody cetuximab, which bears the α-Gal epitope (O'Neil et al., 2007). The major allergenic foods studied in Brazil are associated with fish, egg, milk, wheat, peanut, soy and corn (Boye, 2012). In other parts of the world, bites from tick species *I. ricinus* and *Amblyomma americanum* have been identified as a major cause of this sensitization (Commins et al., 2011; Hamsten et al., 2013a). In Brazil, individuals frequently exposed to *Amblyomma sculptum* may react with IgE production against the tick saliva, as reported for other species, providing some evidence for a link between red meat allergy and tick bites in this part of the world. The related *A. sculptum*, used in this work, was recently classified as one of the species of the *Amblyomma cajennense* complex. This species is widely distributed in central and southern Brazil, Paraguay and northern Argentina, and it is the leading species that humans are frequently exposed to in the study area (Beati et al., 2013; Estrada-Pena et al., 2014; Nava et al., 2014). As ixodid ticks constitute an assorted group of more than 720 species (Barker and Murrell, 2004; Nava et al., 2014), it is common for their parasitism to extend to a wide range of animals including humans. A rural lifestyle, common in Brazil, elevates the risk of exposure to ticks (Farlow et al., 2004), making humans accidental hosts for different species of ticks. We suspect that, similar to *A. americanum*, bites from *A. sculptum* may also produce high titers of IgE and induce anaphylaxis. Here we report the existence of the terminal α-Gal-containing epitope(s) in the saliva of the Brazilian *A. sculptum* tick, and the capacity of this epitope to induce specific IgE antibodies in the α1,3-GalT-KO mouse previously sensitised with injected tick saliva or by the tick bite.

## 2. Materials and methods

### 2.1. Ticks

Tick saliva was obtained by inducing partially and fully engorged adult females of *A. sculptum* to salivate using the pilocarpine induction method (Tatchell, 1967) with modification. Briefly, *A. sculptum* ticks engorging naturally on the horses maintained at the experimental farm of the Federal University of Minas, located at Pedro Leopoldo city, Minas Gerais, Brazil, were carefully harvested, rinsed in distiled water, and fixed to glass microscope slides with double-sided tape. Salivation was induced by injecting 2 μL of pilocarpine (2% in PBS, Sigma–Aldrich, MO, USA) into the hemocoel of the tick using a 50 μL syringe (Hamilton, USA) linked to a manual reoeater dispenser (Hamilton). Ticks were incubated at 35 °C in a humid chamber and saliva was collected with a 10 μL micropipette every 5 min until salivation ceased (2–3 h). Volumes ranged from 2.5 to 50 μL per tick. The total protein content of the saliva was measured by the bicinchoninic acid assay (BCA) method (Protein Reagent kit, Pierce™, USA).

### 2.2. Mice

All animals and experiments were handled in strict accordance with the guidelines of the Research Ethics Committee of the Federal University of Minas Gerais, (UFMG), Belo Horizonte, Brazil and approved under the protocol number 137/2011. Female C57Bl/6 mice (6–8 weeks old), having disrupted alleles of the α1,3-GalT gene (Thall et al., 1995; Milland et al., 2006) (α1,3GalT-KO), were used. These mice have the H-2b genetic background and are bred and maintained at the animal facility of UFMG.

### 2.3. α-Gal antigen linked to Qβ-VLP and conjugate preparation

Qβ-VLPs were prepared and purified as described previously (Hong et al., 2009; Fiedler et al., 2010). All particles were characterised by size-exclusion chromatography, dynamic light scattering (DynaPro, Wyatt Technology, USA), microfluidic gel electrophoresis (Agilent Bioanalyzer 2100, using Protein 80 chips), and electrospray ionization mass spectrometry on an accurate-mass time-of-flight instrument (Agilent G6230B); representative samples were further examined by transmission electron microscopy and multi-angle light scattering (Viscotec, Malvern Instruments, UK). In all cases, standard properties of size and composition were observed, with the particles showing narrow size distributions and high protein purity (less than 5% protein impurities detected). Protein concentrations in solution were measured with the BCA method (Protein Reagent kit, Pierce™, USA), standardised with BSA. For conjugate preparation, α-Gal trisaccharide (α-Gal-OH, Carbosynth US, LLC, San Diego, CA, USA) and glucose were converted to their respective alkyne derivatives by Lewis acid-mediated glycosylation of 3-butyn-2-ol. Each alkyne was attached to Qβ-VLPs by a two-step procedure in which the protein nanoparticle was first acylated with an azide-terminated N-hydroxysuccinimide ester and then addressed by copper-catalysed azide-alkyne cycloaddition.

### 2.4. Mice sensitization for antibody detection

We initially verified the competence of these α1,3-GalT-KO mice, previously immunised, to produce antibodies (primarily IgG) against α-Gal epitopes. Immunisation was performed using the following protocol: a group of 10 mice were s.c. injected with 10 μg per dose, four doses, one per week, of the antigen consisting of the bacteriophage Qβ-VLP to which approximately 540 copies of the Galα3LN epitope ($Q\beta(Gal\alpha3LN)_{540}$) were attached by covalent chemical ligation as described in Section 2.3. A control group of 10 mice was immunised with unmodified Qβ-VLP. Mice were sensitised by tick saliva using two methods. A standard protocol for tick feeding on mice using feeding chambers as described by Bouchard and Wikel in 2005 (Bouchard and Wikel, 2005) was slightly modified, as follows. Groups of 10 mice were anesthetised i.p. with 100 mg/kg of ketamine and 10 mg/kg of xylazine (Uniao Quimica, Brazil), using a tuberculin syringe (BD Safety-Lok™, USA). Once fully anesthetised, a feeding chamber was assembled on each