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Tsetse fly saliva biases the immune response to Th2 and induces anti-vector antibodies that are a useful tool for exposure assessment

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Abstract

Tsetse flies (*Glossina* sp.) are blood-feeding dipteran insects that transmit African trypanosomes, parasites that are responsible for human sleeping sickness and veterinary infections. Increasing attention is being paid to the effects of tsetse fly saliva deposited at the feeding site, which enables the blood-feeding process and putatively promotes parasite transmission. Here we demonstrate that saliva induces strong humoral responses against the major 43–45 kDa protein fraction (tsetse salivary gland proteins 1 and 2 – Tsal1 and Tsal2) in mice and humans and suppresses murine T and B cell responses to heterologous antigen. The saliva-induced immune response is associated with a Th2-biased cytokine profile and the production of mainly IgG1 and IgE antibody isotypes. Functionally, the antibodies raised in mice exposed to tsetse fly bites or induced after experimental saliva immunisation do not affect the fly's blood-feeding efficiency nor its survival. We propose that anti-saliva as well as anti-Tsal1/2 antibody responses can be used in epidemiological studies as a tool to analyze human exposure to tsetse flies.

Keywords: *Glossina*; Vector–host interaction; Immune modulation; T-helper cell type 2; Antibodies

1. Introduction

Tsetse flies (*Glossina* sp.) are obligate haematophagous insects, transmitting the protozoan parasite *Trypanosoma* sp., responsible for disease in livestock and Human African Trypanosomiasis (HAT), also known as sleeping sickness. New infections are initiated when metacyclic trypanosomes are inoculated into the vertebrate skin by the vector during the blood-probing process (Youdeowei, 1975). In natural tsetse populations, *Trypanosoma* sp. prevalence has been estimated to be around 5–10% in endemic regions of African trypanosomiasis. For *Trypanosoma brucei* sp., the causative agents of HAT and nagana in cattle, infection prevalence in flies is much lower (0.1%) (Otieno and Darji, 1979; Morlais et al., 1998; Msangi et al., 1998). As a result, hosts are mostly exposed to the bites of uninfected tsetse flies. It is known that during this interaction, tsetse flies expose the host to a complex mixture of antigens including physiologically active salivary components which overcome host haemostatic responses (Parker and Mant, 1979; Mant and Parker, 1981; Cappello et al., 1996; Champagne, 2004) and are predicted to play a crucial role in the blood-feeding process by ensuring persistent blood flux at the feeding site and preserving mouthpart and crop function by avoiding clot formation. These include a tsetse thrombin inhibitor (TTI, 3.5 kDa) (Cappello et al., 1996), an 11.3 kDa inhibitor of thrombin serine protease and esterase activities (Parker and Mant, 1979) and a >30 kDa protein fraction exerting apyrase activity (Mant and Parker, 1981).

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In addition, several tsetse salivary gland proteins with a yet unknown function have been characterised at the molecular level. These include two related secreted proteins (TSGF-1, 56.6 kDa and TSGF-2, 58.3 kDa) with growth factor and adenosine-deaminase motifs (Li and Aksoy, 2000), tsetse antigen 5 (TAg5, 28.9 kDa) and tsetse salivary gland proteins 1 and 2 (Tsal1, 45.6 kDa and Tsal2, 43.9 kDa) which have been identified as major soluble proteins from *Glossina morsitans morsitans* salivary glands (Li et al., 2001; Haddow et al., 2002). The two abundant homologous gene products Tsal1 and Tsal2 have also been found in *Glossina fuscipes* and *Glossina austeni* and share limited sequence homology with components of the genome of *Anopheles* (GenBank Accession No. EAA03868), the salivary proteome of the house mosquito *Culex* (GenBank Accession No. AAR18449) and the new world sandfly *Lutzomyia* (GenBank Accession No. AAS16916) (Valenzuela et al., 2004). TAg5 shares similarity with the Crisp-Antigen 5 (CAP) family of proteins (Li et al., 2001), including antigen-5 which is an important venom allergen of hornets, wasps and fire ants (Fang et al., 1988; King and Spangfort, 2000). As such, TAg5 might be involved in the hypersensitivity reactions that have previously been observed in tsetse fly-exposed rabbits (Ellis et al., 1986).

The knowledge of the immunological aspects of tsetse fly feeding is very limited. Beside the occasional occurrence of skin reactions, exposure of rabbits to *Glossina palpalis palpalis* or *Glossina morsitans centralis* induces humoral immune responses against several tsetse fly salivary components (Ellis et al., 1986; Matha and Weiser, 1988). In some reported cases, repeated exposure of rabbits to *Glossina* resulted in decreased tsetse feeding efficiencies and decreased longevity (Parker and Gooding, 1979; Matha et al., 1986). However, the authors attributed these effects to locally mediated effects and not to circulating antibodies. The immunogenicity of *Glossina* saliva in other hosts, such as humans, has not been documented so far.

Studies of other haematophagous arthropods demonstrate that salivary components and saliva-specific immune responses play significant roles in the efficiency of blood feeding and disease transmission. For example, components of sandfly saliva bias the host immune response towards a T helper cell type 2 (Th2) environment, favouring the progression of *Leishmania* infection (Titus and Ribeiro, 1988; Lima and Titus, 1996; Belkaid et al., 1998). In that context, the vasodilatory peptide maxadilan with anti-inflammatory properties (Morris et al., 2001) and Salp15 as suppressor of T helper cell activation (Anguita et al., 2002) were identified as active immune modulatory and putative transmission-promoting constituents of sandfly saliva. Pre-exposure of mice to sandflies (Kamhawi et al., 2000; Thiakaki et al., 2005) as well as immunisation against individual salivary components (Morris et al., 2001; Valenzuela et al., 2001) were shown to provide partial or complete protective immunity against natural *Leishmania* transmission. This protection is associated with the induction of a strong delayed-type hypersensitivity (DTH) response at the site of parasite delivery. Additionally, the antibody responses to sandfly salivary antigens could be used to monitor host exposure to this medically important vector and provide a suitable risk marker for *Leishmania* transmission in endemic areas (Rohousova et al., 2005). Moreover, natural or experimental host anti-sandfly immunisation reduced blood-feeding efficiency and increased mortality in the post-bloodmeal vector population (Ghosh and Mukhopadhyay, 1998).

In the case of *Glossina*, comparable studies of the immune modulatory potential of saliva have yet to be undertaken. Host anti-*Glossina* immunity has been addressed only in experimental settings, while the effects on vector fitness remain unclear. Therefore, this study focuses on the immune response raised against tsetse fly saliva in mice and humans as well as the impact of naturally and experimentally induced anti-vector immune responses on the blood-feeding efficiency and survival of tsetse flies. Additionally, this study evaluated whether anti-saliva humoral responses as well as responses against individual recombinant proteins can be used to analyze tsetse fly exposure in epidemiological studies and as such function as a risk measure for trypanosomiasis.

2. Materials and methods

2.1. Animals

Six- to eight-week-old female in-house bred F1 (BALB/c × C57BL/6) mice were used for all experiments. Mouse care and experimental procedures were performed under approval from the Ethical Committee of the Vrije Universiteit Brussel. Tsetse flies (*G. m. morsitans*) were available from the insectaria at the Prins Leopold Institute of Tropical Medicine Antwerp (ITMA), originating from puparia collected in Kariba (Zimbabwe) and Handeni (Tanzania). Flies were fed on rabbits and maintained at 26 °C and at a relative humidity of 65%. Animal Ethics approval for the tsetse fly feeding on live animals was obtained from the Animal Ethical Commission of the Institute of Tropical Medicine, Antwerp (Belgium).

2.2. Tsetse fly saliva isolates

Three days after the last blood meal, 10- to 15-day-old tsetse flies were dissected and salivary glands were isolated under a binocular microscope using forceps and collected in ice-cold, sterile PBS (pH 7.4). Centrifugation for 1 min at 20,000*g* separated the salivary gland tissue in the pellet and soluble salivary components in the supernate (= saliva), with an approximate yield of 10 μg per fly (5 μg/gland). Saliva was subsequently sterilised by filtration through a 0.2 μm pore sized filter and lipopolysaccharides were removed using Remtox beads. Protein concentrations were assessed by the BCA protein assay reagent kit (Pierce Biotechnology) and aliquots were stored at −20 °C.