INSTITUT PASTEUR

Microbes and Infection 9 (2007) 1454–1462

www.elsevier.com/locate/micinf

Original article

Antibody response against saliva antigens of *Anopheles gambiae* and *Aedes aegypti* in travellers in tropical Africa

Eve Orlandi-Pradines [a,b], Lionel Almeras [a,b], Laure Denis de Senneville [a,b], Solenne Barbe [a,b], Franck Remoué [c], Claude Villard [d], Sylvie Cornelie [c], Kristell Penhoat [a,b], Aurélie Pascual [a,b], Catherine Bourgouin [e], Didier Fontenille [f], Julien Bonnet [f], Nicole Corre-Catelin [g], Paul Reiter [g], Frederic Pagés [h,b], Daniel Laffite [d], Denis Boulanger [c], François Simondon [c], Bruno Pradines [a,b], Thierry Fusaï [a,b], Christophe Rogier [a,b,*]

[a] *Unité de recherche en biologie et en épidémiologie parasitaires, Institut de Médecine Tropicale du Service de Santé des Armées, Parc le Pharo, BP 46, 13998 Marseille-Armées, France*

[b] *Institut fédératif de la recherche 48, IFR48, Marseille, France*

[c] *Institut de Recherche pour le développement (IRD), UR24 «Epidémiologie et Prévention» Unit, Centre IRD de Hann, BP 1386, Dakar, Senegal*

[d] *Plate-forme de protéomique, IFR125, Faculté de Pharmacie, 27 Bv Jean-Moulin, 13385 Marseille, France*

[e] *Centre de production et d'infection des Anophèles (CEPIA), Institut Pasteur, 25–28 rue du Dr. Roux, 75724 Paris Cedex 15, France*

[f] *Institut de Recherche pour le développement (IRD), UR16 ''caractérisation et contrôle des populations de vecteurs'' Unit, Centre IRD, BP 64501, Montpellier 34394 Cedex 5, France*

[g] *Insectes et Maladies infectieuses, Institut Pasteur, 25-28 rue du Dr. Roux, 75724 Paris Cedex 15, France*

[h] *Unité d'Entomologie Médicale, Département d'épidémiologie et de santé publique Sud, Institut de Médecine Tropicale du Service de Santé des Armées, Parc le Pharo, BP 46, 13998 Marseille-Armées, France*

Received 16 April 2007; accepted 31 July 2007
Available online 7 August 2007

Abstract

Exposure to vectors of infectious diseases has been associated with antibody responses against salivary antigens of arthropods among people living in endemic areas. This immune response has been proposed as a surrogate marker of exposure to vectors appropriate for evaluating the protective efficacy of antivectorial devices. The existence and potential use of such antibody responses in travellers transiently exposed to *Plasmodium* or arbovirus vectors in tropical areas has never been investigated. The IgM and IgG antibody responses of 88 French soldiers against the saliva of *Anopheles gambiae* and *Aedes aegypti* were evaluated before and after a 5-month journey in tropical Africa. Antibody responses against *Anopheles* and *Aedes* saliva increased significantly in 41% and 15% of the individuals, respectively, and appeared to be specific to the mosquito genus. A proteomic and immunoproteomic analysis of anopheles and *Aedes* saliva allowed for the identification of some antigens that were recognized by most of the exposed individuals. These results suggest that antibody responses to the saliva of mosquitoes could be considered as specific surrogate markers of exposure of travellers to mosquito vectors that transmit arthropod borne infections.

Keywords: Malaria; Arbovirus; Vectors; Saliva; Antibody response; Proteomic; Travellers; *Anopheles gambiae*; *Aedes aegypti*

Abbreviations: SD, standard deviation; Ig, immunoglobulin; ELISA, enzyme-linked immunosorbent assay; PBS, phosphate-buffered saline; OD and aOD, optical density and adjusted optical density; HRP, horse radish peroxidase; MS and MS/MS, mass spectrometry and tandem mass spectrometry; MALDI–TOF, matrix assisted laser desorption ionization–time-of-flight; PMF, peptide mass fingerprinting; 95% CI, 95% confidence interval; SAAng, saliva antigens of *An. gambiae*; SAAea, saliva antigens of *Ae. aegypti*.

* Corresponding author. Unité de recherche en biologie et épidémiologie parasitaires, Institut de Médecine Tropicale du Service de Santé des Armées, Bd. Charles Livon, Parc le Pharo, BP 46, 13998 Marseille Armées, France. Tel.: +33 4 91 15 01 50; fax: +33 4 91 15 01 64.

E-mail address: christophe.rogier@wanadoo.fr (C. Rogier).

1286-4579/$ - see front matter © 2007 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.micinf.2007.07.012

1. Introduction

Malaria and arbovirosis, like dengue or yellow fever, are major threats in inter-tropical areas and responsible for 2–3 million deaths and 500 000 cases, respectively (WHO, http://www.who.int). Their agents are transmitted by mosquito vectors, like *Anopheles gambiae* for *Plasmodii* and *Aedes aegypti* for some arbovirus. Individual antivectorial devices like impregnated bed-nets, repellents and long-sleeved clothes are proposed to protect non-immune people travelling across endemic areas against mosquitoes vectors. However, the use of these antivectorial devices is often perceived as restrictive, and their efficacy in protecting travellers has not been thoroughly evaluated. Entomological methods are not designed for estimating individual exposure level to mosquito vector and their use is not practical for the evaluation of the efficacy of antivectorial devices among travellers.

In contrast, serological methods may provide an indirect means of estimating individual exposure to mosquito vectors. Several studies have shown that mosquito saliva is immunogenic. Early studies have demonstrated that allergic persons develop immunoglobulin G4 (IgG4) and IgE to mosquito saliva antigens [1–4]. Recently, studies have demonstrated that people living in malaria endemic areas, who are constantly exposed to anopheles bites, develop antibodies to anopheles salivary proteins [5,6]. *An. gambiae* anti-saliva IgG antibody responses have been shown to represent an immunological indicator of the level of *P. falciparum* transmission [6]. Association of the level of specific antibody responses to the saliva of arthropods with exposure to the corresponding vectors has also been demonstrated for Lyme disease [7], Chagas disease [8], leishmaniasis [9] and African trypanosomiasis [10]. These studies have focused on populations living in endemic areas, never on travellers transiently exposed to vectors. Therefore, it is unclear whether the saliva antigens of the main malaria and arbovirus vectors are immunogenic enough to trigger a detectable antibody response after only a few bites.

The objectives of this study were: (i) to analyse the development of antibody responses against saliva antigens of *An. gambiae* and *Ae. aegypti* in travellers who are transiently exposed to these vectors in endemic areas of tropical Africa; (ii) to evaluate the kinetics of these responses; and (iii) to characterize the antigenic saliva proteins from these two mosquito species. The antibody responses against the saliva antigens of mosquitoes could be analysed as a surrogate serological marker of individual exposure to vector bites in persons who are only transiently exposed and used for the evaluation of the efficacy of antivectorial devices among travellers.

2. Materials and methods

2.1. Population studied

The anti-saliva antibody response has been studied in 88 French soldiers (male, age mean ± SD: 24.3 ±4.1 years, Caucasians) who travelled during a 5-month period in tropical Africa (Gabon and Côte d'Ivoire) where *An. gambiae* and *Ae. aegypti* are endemic [11,12]. Among them, 48 had previously travelled in countries where at least one of these mosquitoes is endemic. During their mission, they lived 64 and 36 days in urban and rural areas of Gabon, respectively, and 15 and 53 days in urban and rural areas of Côte d'Ivoire, respectively. The rural conditions in Côte d'Ivoire were mostly rough and they often slept in damaged houses or bivouacs. Blood samples were collected before their departure from France (T1), after 2 months in tropical Africa (T2), at the end of their mission (T3) and 3 months after returning to France (T4). Blood samples were obtained by venous puncture, centrifuged and sera were stored at −20 °C. In total, 88 and 87 blood samples were available for serological analysis at T1 and T3, respectively. Due to difficulties in obtaining saliva antigens, ELISA tests were done in a restricted number of randomly chosen samples: 35 and 33 samples for IgG response against saliva antigens of *An. gambiae* (SAAng) at T2 and T4, respectively, 41 pairs of sera for anti-SAAng IgM at T1 and T3, and 48 pairs of sera for IgG response against saliva antigens of *Ae. aegypti* (SAAea) at T1 and T3.

The volunteers used anti-malaria chemoprophylaxis (100 mg doxycycline per day during the mission and 4 weeks after its end) and individual antivectorial devices like impregnated bed-nets, repellents and long-sleeved battle dresses provided by the Army.

Blood samples were obtained twice at a 3–5-month intervals from 22 individuals living permanently in Marseille (France), i.e. an area free of *An. gambiae* or *Ae. aegypti*, and who had never travelled in countries that are endemic for these mosquitoes.

All participants gave their informed consent to take part in the study and the protocol was approved by Marseille-2 Ethical Committee.

2.2. Method of mosquito salivation

The *An. gambiae* and *Ae. aegypti* used in this study were the YAOUNDE and BORA-BORA reference strain, respectively, maintained under standard conditions. The mosquito salivation technique was performed as described by Remoue et al. [6]. Total protein concentrations was estimated from the absorbance values at 280 and 260 nm, using the formula $1.55 \times A_{280} - 0.76 \times A_{260}$, and the concentration adjusted at 400 µg/ml for *Anopheles* and 200 µg/ml for *Aedes*. Mosquito saliva samples were pooled, aliquoted in 100 µl (corresponding to 20 mosquitoes), lyophilized and stored at −20 °C without any anti-protease cocktail.

2.3. Enzyme-linked immunosorbent assay

The sera were tested by ELISA for the presence of IgG and IgM antibodies that bind to saliva. Mosquito saliva (1 µg/ml in phosphate-buffered saline (PBS)) was coated on flat-bottom microtitre plates maxisorb (Nunc, Denmark) overnight at 4 °C. Thereafter, the plates were blocked for 2 h at 37 °C with 200 µl of blocking buffer consisting of PBS solution plus 0.1% Tween-20 (Sigma Chemical Co., USA) and 3%