Vaccine 29 (2011) 77–82

Contents lists available at ScienceDirect

Vaccine

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

# Characterization of *Aedes albopictus* akirin for the control of mosquito and sand fly infestations

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ARTICLE INFO

*Article history:*
Received 12 May 2010
Received in revised form
24 September 2010
Accepted 6 October 2010
Available online 20 October 2010

*Keywords:*
Akirin
Arthropod
Mosquito
Sand fly
Subolesin
Tick
Vaccine

ABSTRACT

The control of arthropod vectors of pathogens that affect human and animal health is important for the eradication of vector-borne diseases. Recent evidences showed a reduction in the survival and/or fertility of mosquitoes, sand flies and poultry red mites fed in vitro with antibodies against the recombinant *Aedes albopictus* akirin. These experiments were the first step toward the development of a multi-target arthropod vaccine. In this study, we showed that the oviposition of *A. albopictus* and *Phlebotomus perniciosus* fed on mice vaccinated with recombinant *A. albopictus* akirin was reduced by 17% and 31%, respectively when compared to controls. However, *Aedes aegypti* mosquitoes were not affected after feeding on vaccinated mice. These results showed that recombinant *A. albopictus* akirin could be used to vaccinate hosts for the control of mosquito and sand fly infestations and suggested new experiments to develop improved vaccine formulations.



## 1. Introduction

Diseases caused by vector-borne pathogens greatly impact human and animal health, accounting for over 20% of all emerging infectious diseases recorded between 1940 and 2004 [1]. In particular, insects such as mosquitoes and sand flies are considered the most important vectors of human diseases worldwide [1]. *Aedes* spp. (Diptera: Culicidae) are vectors of many viruses that cause human disease, including the exposure of over 2.5 billion people in tropical and subtropical countries to dengue fever virus [2]. Phlebotomine sand flies (Diptera: Psychodidae) are also vectors of several pathogens including *Leishmania* spp., which causes various forms of human leishmaniasis [3].

With the exception of a few diseases such as yellow fever, vaccines against vector-transmitted pathogens have not been successfully developed nor implemented, and intense use of insecticides and/or chemotherapy have resulted in an increasing number of insecticide-resistant vectors and drug-resistant pathogens [4–6].

The effect of anti-tick vaccines on the reduction of tick infestations and the transmission of some tick-borne pathogens [4,7,8] and preliminary results obtained in insect vector species [9–17] have provided evidence that protective antigens may be used for development of vaccines with the dual target control of both arthropod infestations and reduction of vector capacity to transmit pathogens that impact human and animal health.

Subolesin, the ortholog of akirin in ticks, was discovered as a tick protective antigen in *Ixodes scapularis* [18]. Subolesin was shown by RNAi gene knockdown and immunization trials using the recombinant protein to protect hosts against tick infestations, reduce tick survival and reproduction, cause degeneration of guts, salivary glands, reproductive tissues and embryos and to decrease the vector capacity of ticks for *Anaplasma marginale* and *A. phagocytophilum* [18–24]. In addition, subolesin was shown to be evolutionary conserved and similar in structure and function to insect and vertebrate akirins, which control NF-kB-dependent

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doi:10.1016/j.vaccine.2010.10.011

and independent gene expression that impact innate immunity [21,25–28].

Recent evidences showed a reduction in the survival and/or fertility of mosquitoes, sand flies and poultry red mites fed in vitro with antibodies against the recombinant *Aedes albopictus* akirin [17,29]. Vaccination with recombinant *A. albopictus* akirin also reduced tick infestations [17,30]. These results suggested the experiments reported here to characterize the effect of *A. albopictus* akirin for the control of mosquito and sand fly infestations in mice vaccinated with the recombinant protein. These results support the role of mosquito akirin as a protective antigen for the control of mosquito and sand fly infestations.

## 2. Materials and methods

### 2.1. Mosquitoes and sand flies

Mosquitoes, *A. albopictus*, from the Baix Llobregat (Barcelona, Spain) colony were used to establish a colony at the University of Zaragoza and used for this study. The *A. albopictus* strain has been maintained in the laboratory for approximately one year (12 generations). Mosquitoes were reared in an environmental cabinet at 25 ± 1 °C, 75 ± 5% relative humidity (RH), and a 12 h light:12 h dark photoperiod.

Mosquitoes, *Aedes aegypti*, Bora-Bora reference strain originating from Bora-Bora (French Polynesia) were used for this study. The *A. aegypti* strain has been maintained in the laboratory for 12 years (approximately 150 generations). Mosquitoes were maintained in the IRD-Monpellier insectary under controlled conditions of 27 ± 2 °C, 80% RH and a 17 h light:7 h dark photoperiod.

Sand flies, *Phlebotomus perniciosus*, used in this study were from an autochthonous (Madrid Province, Spain) colony established at the Medical Entomology Unit of the Instituto de Salud Carlos III (Spain). The *P. perniciosus* strain has been maintained in the laboratory for approximately 161 generations. Flies were reared in an environmental cabinet at 28 °C, 95–100% RH, and a 17 h light:7 h dark photoperiod.

For all insect species, females were mixed with males after emergence until females were selected for the experiment. Under our rearing conditions, most of the insects mate as demonstrated by ovaries dissection and examination in previous experiments. After emergence, insects were supplied absorbent cotton saturated with 20–30% sucrose solution during four days. At day five, they were starved 5 h before mice infestation [31]. Then, females were maintained in new cages without males until the end of the experiment.

### 2.2. Vaccine formulation

The expression and purification of recombinant *A. albopictus* akirin was conducted as previously reported using an extractive bioconversion process in an aqueous two-phase system supporting *Pichia pastoris* growth and protein secretion [32]. Recombinant antigen or saline were adjuvated in Montanide ISA 50 V2 (Seppic, Paris, France).

### 2.3. Mouse vaccination and infestation

Three experiments were conducted to evaluate the effect of recombinant *A. albopictus* akirin vaccination on mice infested with *A. albopictus*, *A. aegypti* and *P. perniciosus*. These experiments were independently conducted at the University of Zaragoza (Spain), the Centre IRD de Montpellier (France) and the Instituto de Salud Carlos III (Spain), respectively. All mice were immunized three times at weeks 0, 3 (4 for the experiment with *A. aegypti*), and 6 with 1 ml (0.2 ml for the experiment with *P. perniciosus*) doses injected subcutaneously in the dorsum near the base of the tail using a 1-ml tuberculin syringe and a 27.5-G needle. Vaccinated and control mice were injected with recombinant *A. albopictus* akirin (50 μg/dose) and adjuvant/saline, respectively. Immunizations, insect collections and evaluations were done blinded and the key to the experimental groups was not opened until the end of the experiment. Animals were cared for in accordance with standards specified in the Guide for Care and Use of Laboratory Animals and approved by ethical committees for animal care and experimentation.

#### 2.3.1. Vaccination against A. albopictus mosquito infestations

Five female 5 weeks old Balb/c mice were used for each of the vaccinated and control groups. Each mouse was restrained in a nylon cage, and exposed to *A. albopictus* bites at week 8 of the experiment. Twenty female mosquitoes aged 3–5 days old were fed on each mouse during 30 min. After exposure, fed females were transferred to individual vials for oviposition. Eggcups were removed seven days post-infestation and eggs were counted. The mortality rate seven days post-infestation was also evaluated. Four weeks after the last immunization, blood samples were collected from each mouse.

#### 2.3.2. Vaccination against A. aegypti mosquito infestations

Four female 8–10 weeks old Balb/c mice were used for each of the vaccinated and control groups. Infestations were done at week 8. Each mouse was restrained in a perforated tube, placed on a warming plate and exposed to *A. aegypti* bites during 15 min. Blood samples were collected approximately 48 h before each immunization and infestation, and seven days after infestation by saphenous vein or retro-orbital bleeding. Thirty female mosquitoes were fed on each mouse during 15 min. An oviposition cup was placed into each cage and females were allowed to oviposit. Eggcups were removed seven days post-infestation and eggs were counted. The mortality rate seven days post-infestation was also evaluated.

#### 2.3.3. Vaccination against sand fly infestations

Five female 6–8 weeks old CD-1 mice were used for each of the vaccinated and control groups. The effect on mortality and fertility of *P. perniciosus* sand flies fed on vaccinated and control mice was evaluated. Two weeks after the last immunization, 25 female sand flies were fed for 20–30 min on each mouse anaesthetized with a blend of ketamine (100 mg/kg) and xylazine (10 mg/kg) by intraperitoneal injection [33]. After sand fly exposure, 10 fed females per group were transferred to individual flasks for analysis of oviposition. Eggs from females fed on the same mouse were transferred to a pot for larval rearing to study the development to adult flies as described previously [17]. Four weeks after the last immunization, blood samples were collected from each mouse.

### 2.4. Characterization of the immune response in immunized mice by ELISA

Serum was separated in blood samples by centrifugation and individually stored at −20 °C. An indirect ELISA test was performed to detect antibodies against akirin in mouse serum samples. High absorption capacity polystyrene microtiter plates were coated with 50 μl (0.02 μg/ml solution of purified akirin; [32]) per well in carbonate–bicarbonate buffer (Sigma, Barcelona, Spain). After an overnight incubation at 4 °C, coated plates were blocked with 200 μl/well of blocking solution (5% skim milk in PBS). Serum samples or PBS as negative control were diluted (1:20, v/v) in blocking solution and 100 μl/well were added into duplicate wells of the antigen-coated plates. After a 1 h incubation period at RT, the plates were washed three times with a washing solution (PBS containing 0.05% Tween 20). A goat anti-mouse IgG-peroxidase conjugate